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# **Deuterium labelling and rearrangement studies of lignans**

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ACADEMIC DISSERTATION

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# Abstract

Lignans are naturally occurring compounds, polyphenolic secondary plant and mammalian metabolites. Due to their ubiquitous presence and biological activity, lignans have attracted the interest of scientists from different areas, like nutrition scientists, pharmaceutical researchers and synthetic chemists. The research is very active, and the number of lignan related publications has proliferated.

Lignans vary widely in the structure, and the present work focuses mainly on the (hydroxy)lignano-9,9'-lactones, their rearranged products, and 9,9'-epoxylignanes. The literature review introduces the stereochemistry and assignment of the absolute configuration of these lignans. In addition, stable isotope labelling of lignans is reviewed.

The experimental part is focused on deuteration of lignans and rearrangement and stereochemistry studies. The deuteration reaction utilising acidic H/D exchange within the lignan skeleton was investigated. The relative reactivity of various aromatic sites, the stability of deuterium labels and the isotopic purity of the labelled compounds were examined. Experimental observations and results were compared to computational studies. Several stable, isotopically pure polydeuterated lignano-9,9'-lactones and 9,9'-epoxylignanes were synthesised. Alongside the deuteration experiments unexpected reactivity in electrophilic aromatic deuteration of methylenedioxy substituted compounds was observed and further studied.

In addition to deuteration, the stereochemistry of certain rearranged lignanolactones was a central subject of this study. Our findings allowed to clarify some mechanistical aspects of the rearrangement reactions of 7'-hydroxylignano-9,9'-lactones and revise certain disputable structural data in the literature. Furthermore, the X-ray structures of 7'-hydroxylignano-9,9'-lactones and rearranged 9'-hydroxylignano-9,7'-lactones were obtained for the first time.

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Helsinki, August 2014  
Monika Pohjoispää

# List of original publications

This thesis is based on the following original publications:

- I** Barbara Raffaelli, Monika Pohjoispää, Tapio Hase, Christine J. Cardin, Yu Gan and Kristiina Wähälä. Stereochemistry and rearrangement reactions of hydroxylignan lactones. *Org. Biomol. Chem.* **2008**, 6, 2619–2627. doi: 10.1039/b801593g
- II** Monika Pohjoispää, Barbara Raffaelli, Yu Gan, Christine J. Cardin and Kristiina Wähälä. Structures of the plant lignan (–)-parabenzlactone and its rearranged regioisomer. Manuscript, **2014**.
- III** Eija Leppälä, Monika Pohjoispää, Jorma Koskimies and Kristiina Wähälä. Synthesis of new deuterium-labelled lignan lactones. *J. Label. Compd. Radiopharm.* **2008**, 51, 407–412. doi: 10.1002/jlcr.1553
- IV** Monika Pohjoispää, Raúl Mera-Adasme, Dage Sundholm, Sami Heikkinen, Tapio Hase and Kristiina Wähälä. Capricious selectivity in electrophilic aromatic deuteration of methylenedioxy substituted compounds. Manuscript, **2014**.
- V** Monika Pohjoispää and Kristiina Wähälä. Synthesis of 3,4-dibenzyltetrahydrofuran lignans (9,9'-epoxylignanes). *Molecules* **2013**, 18, 13124–13138. doi: 10.3390/molecules181113124

The publications are referred to by Roman numerals (I–V). The published articles are reproduced in the printed version of this thesis with the kind permission of the publishers.

# Abbreviations

Ac	acetyl
aq.	aqueous
Ar	aryl
bmim	1-butyl-3-methylimidazolium
Bn	benzyl
Bu, <i>n</i> -Bu	butyl, <i>normal</i> butyl
<i>t</i> -Bu	<i>tert</i> -butyl
COSY	correlation spectroscopy
d.e.	diastereomeric excess
DMF	<i>N,N</i> -dimethylformamide
DMI	1,3-dimethyl-2-imidazolidinone
DMPU	1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone
DP	dirigent protein
e.e.	enantiomeric excess
EI	electron ionisation (or electron impact)
ESI	electrospray ionisation
ESP	electrostatic potential
Et	ethyl
GC	gas chromatography
Glu	glucoside
(HP)LC	(high-performance) liquid chromatography
ID	isotope dilution
ip	isotopic purity
IUPAC	International Union of Pure and Applied Chemistry
IR	infrared
LC	liquid chromatography
LDA	lithium diisopropylamide
LHMDS	lithium hexamethyl disilazide
Me	methyl
MS	mass spectrometry
MW	microwave
NMR	nuclear magnetic resonance
NOESY	nuclear overhauser effect spectroscopy
Piv	pivaloyl
Ph	phenyl
Py	pyridine
rt	room temperature
SID	stable isotope dilution
SIM	selected ion monitoring
TBAF	tetra- <i>n</i> -butylammonium fluoride
TBDMS	<i>tert</i> -butyldimethylsilyl
THF	tetrahydrofuran
TIPS	triisopropylsilyl
TMEDA	tetramethylethylenediamine
TMS	trimethylsilyl
Ts	tosyl

# Contents

<b>Abstract .....</b>	<b>3</b>
<b>Acknowledgements .....</b>	<b>4</b>
<b>List of original publications .....</b>	<b>5</b>
<b>Abbreviations .....</b>	<b>6</b>
<b>Contents .....</b>	<b>7</b>
<b>1 Introduction to lignans .....</b>	<b>9</b>
1.1 Structure of lignans .....	10
1.2 Classification .....	11
1.3 Numbering and nomenclature .....	13
<b>2 Absolute configuration of lignans .....</b>	<b>14</b>
2.1 Stereochemistry of lignans .....	14
2.2 Stereochemistry at carbons 8 and 8' .....	15
2.3 Stereochemistry at carbons 7 and 7' .....	18
2.4 Stereochemistry of 9'-hydroxylignano-9,9'-lactones .....	20
<b>3 Biological properties of lignans .....</b>	<b>26</b>
3.1 Lignan biosynthesis .....	26
3.2 Mammalian lignans .....	30
3.3 Dietary lignans .....	32
3.4 Metabolism of dietary lignans .....	33
<b>4 Stable isotope labelling and lignans .....</b>	<b>36</b>
4.1 Stable isotope labelled compounds .....	36
4.1.1 Requirements for the internal standards .....	37
4.1.2 Labels for lignans .....	38
4.2 Synthesis of deuterated lignans .....	40
4.2.1 Introduction of deuteriums via reduction .....	40
4.2.2 Labelling via H/D exchange of aromatic protons .....	42
<b>5 The aims of the present study .....</b>	<b>46</b>
<b>6 Results and discussion .....</b>	<b>47</b>
6.1 Synthesis of lignans .....	47
6.2 Synthesis of deuterium labelled lignans .....	49
6.2.1 Synthesis of new deuterium labelled lignano-9,9'-lactones using D <sub>3</sub> PO <sub>4</sub> ·BF <sub>3</sub> /D <sub>2</sub> O .....	49
6.2.2 Synthesis of deuterium labelled lignano-9,9'-lactones and 9,9'- epoxylignanes using DCl/D <sub>2</sub> O under microwave heating .....	51
6.2.3 Deuteration of dehydroxycubebin .....	53

6.2.4	Characterisation of the deuterated compounds.....	56
6.2.5	H/D exchange reactions for lignans .....	59
<b>6.3</b>	<b>Rearrangement and stereochemistry studies .....</b>	<b>60</b>
6.3.1	Translactonisation studies with oxygen-18 labelled compounds.....	60
6.3.2	Translactonisation studies with KOH/MeOH and NaH/DMF.....	64
6.3.3	Crystal structures of (–)-parabenzlactone and rearranged regioisomer... ..	66
6.3.4	Discussion on the all- <i>trans</i> and all- <i>cis</i> isomers of rearranged hydroxymatairesinol .....	67
<b>7</b>	<b>Conclusions .....</b>	<b>72</b>
<b>8</b>	<b>Experimental .....</b>	<b>73</b>
	<b>References.....</b>	<b>75</b>
	<b>Appendix.....</b>	<b>89</b>

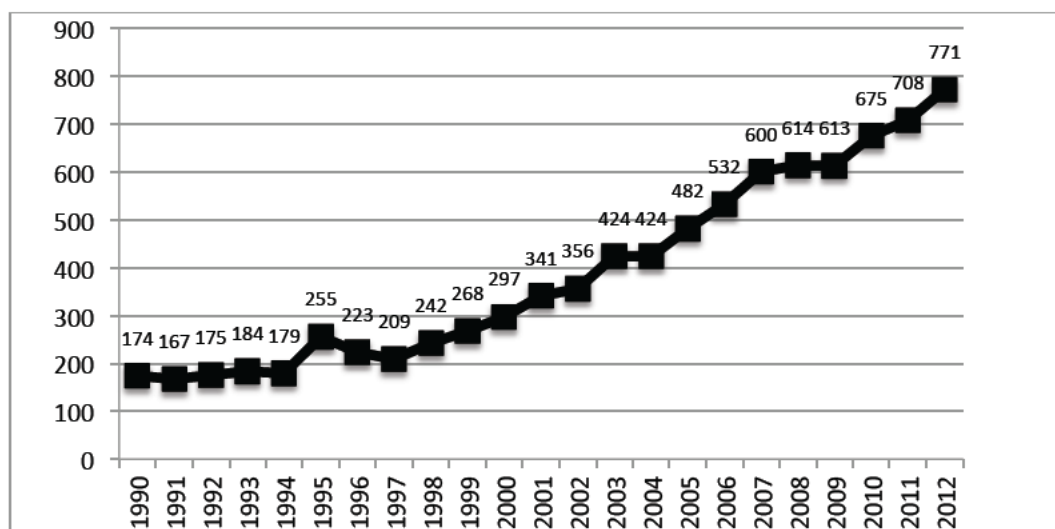


# 1 Introduction to lignans

Lignans are a large and ubiquitous class of natural products, one of the oldest known and used as folk remedies by many different cultures. Initially they have aroused interest due to their widespread occurrence in the plant kingdom and their diverse medically important biological properties. Later new lignans were found also in mammals, resulting from metabolic conversion of dietary lignans.

The early literature on lignans considered mainly the isolation, synthesis and derivatisation of naturally occurring plant lignans. When the first mammalian lignans were reported simultaneously in Finland and the United Kingdom in 1980,<sup>1,2</sup> the lignans suddenly started to interest a wide range of scientist, not only the wood and natural product chemists or botanists, but also food chemists and nutrition scientists, medicinal researchers, pharmaceutical and food industry. Until 1979 less than 700 articles in total had been published on lignans. Now, 30 years later the annual amount of lignan papers is more than that (Figure 1).

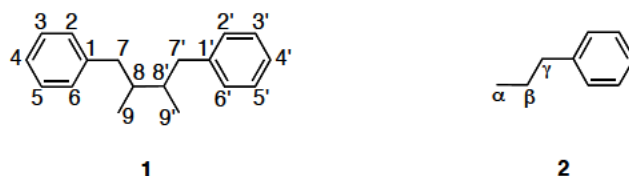
There are numerous reviews published on lignan chemistry<sup>3,4,5,6</sup> in addition to the two early books focused on lignans: a compilation of experts' reviews edited by Rao in 1978,<sup>7</sup> and a comprehensive monograph of Ayres and Loike in 1990.<sup>8</sup> For example Umezawa, Davin and Lewis with co-workers have widely studied and reviewed lignan biosynthesis.<sup>9,10,11,12,13,14</sup> A series of reviews by Whiting<sup>15,16,17</sup> and Ward<sup>18,19,20,21</sup> cover the literature on lignans, neolignans and related compounds up until December 1997. Likely due to the continuously increasing number of publications, the later reviews are, instead of being general, more focused on e.g. different lignan structures, synthetic strategies or lignans possessing biological activity.<sup>22,23,24</sup>



**Figure 1** Number of published lignan related articles per year from 1990. (Data from SciFinder Scholar, September 2013, keyword: lignan.)

## 1.1 Structure of lignans

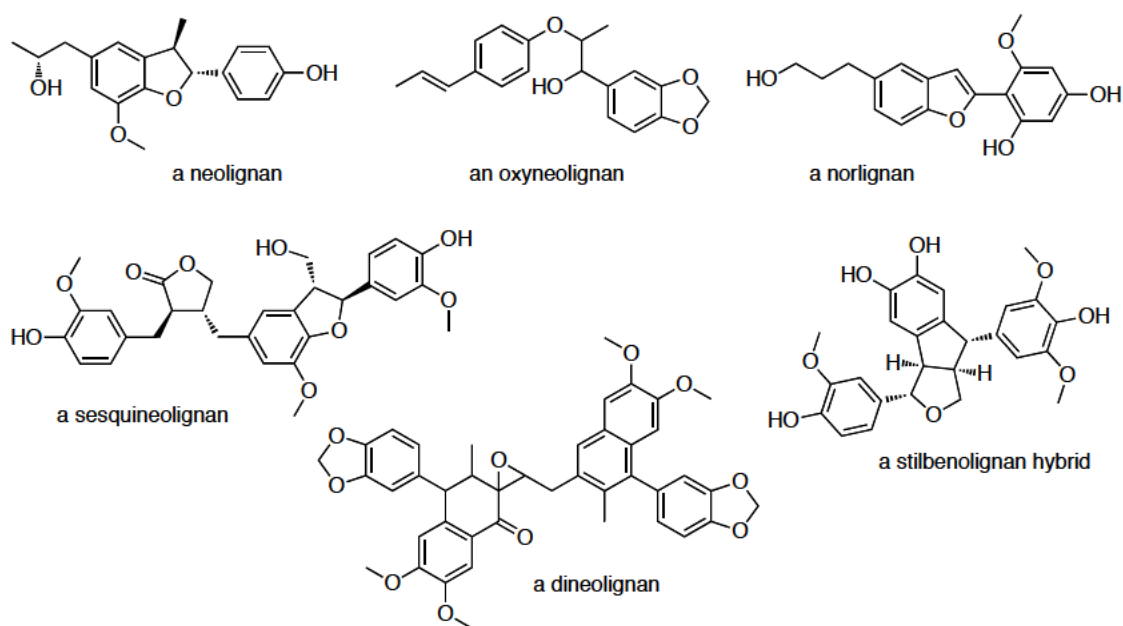
In a review of natural resins in 1936, Haworth introduced the name lignane to describe compounds possessing a skeleton structure **1** derived from two phenylpropanoid unit **2** connected at the central carbons (C-8) of the side chains by a so-called  $\beta$ - $\beta'$ -linkage (Figure 2).<sup>25</sup>



**Figure 2** The definition and a parent structure of lignan **1** formed by  $\beta$ - $\beta'$ -linkage of two  $C_6C_3$  units **2**. For the purposes of numbering the various parent structures, the  $C_6C_3$  unit (**2**) is numbered 1 to 9, where the  $\alpha$  position is 9,  $\beta$  is 8 and  $\gamma$  is 7.<sup>26</sup>

The group was extended in 1972 by Gottlieb, who coined the term neolignan for phenylpropanoid dimer compounds linked in a manner other than C8-C8' (Figure 3).<sup>27</sup> Later the definition was revised to include only dimers of propenyl- and/or allylphenylmonomers.<sup>28</sup> Unfortunately, the various classifications have led to a confusing application of these terms in the literature. Also varying numbering systems of the compound skeleton as well as wide use of different trivial names has made it challenging to follow the advances in this field.<sup>15,28,29,30</sup> The growing interest in lignans and neolignans and the increasing number of variations of their frameworks made it inevitable to create a rational system for naming and numbering these compounds. Thus an IUPAC recommendation on the nomenclature of lignans and neolignans was published in 2000.<sup>26</sup>

The discussion on classification and nomenclature of lignans and related compounds is ongoing.<sup>12</sup> In this thesis the nomenclature that is mostly applied at present is used (Figure 3). The general division for the phenylpropanoid dimers are lignans, neolignans and oxyneolignans. As mentioned, in lignans the two  $C_6C_3$  monomers are linked by a bond between carbons 8 and 8'. In neolignans the two  $C_6C_3$  units are linked by some other carbon-carbon bond, and in oxyneolignans there are no direct carbon-carbon bonds between the monomers which are linked by an ether oxygen atom. Structures with more than two  $C_6C_3$  units are oligomeric lignans, such as sesqueneolignans with three  $C_6C_3$  units, dineolignans with four  $C_6C_3$  units, and sesterneolignans with five  $C_6C_3$  monomers. The prefix *nor-* is used for structures that resemble lignans, neolignans or oxyneolignans, but carry one less carbon atom in the skeleton. When a lignan is attached to other compounds, such as terpenes, stilbenes or flavonoids, it is called a hybrid lignan.

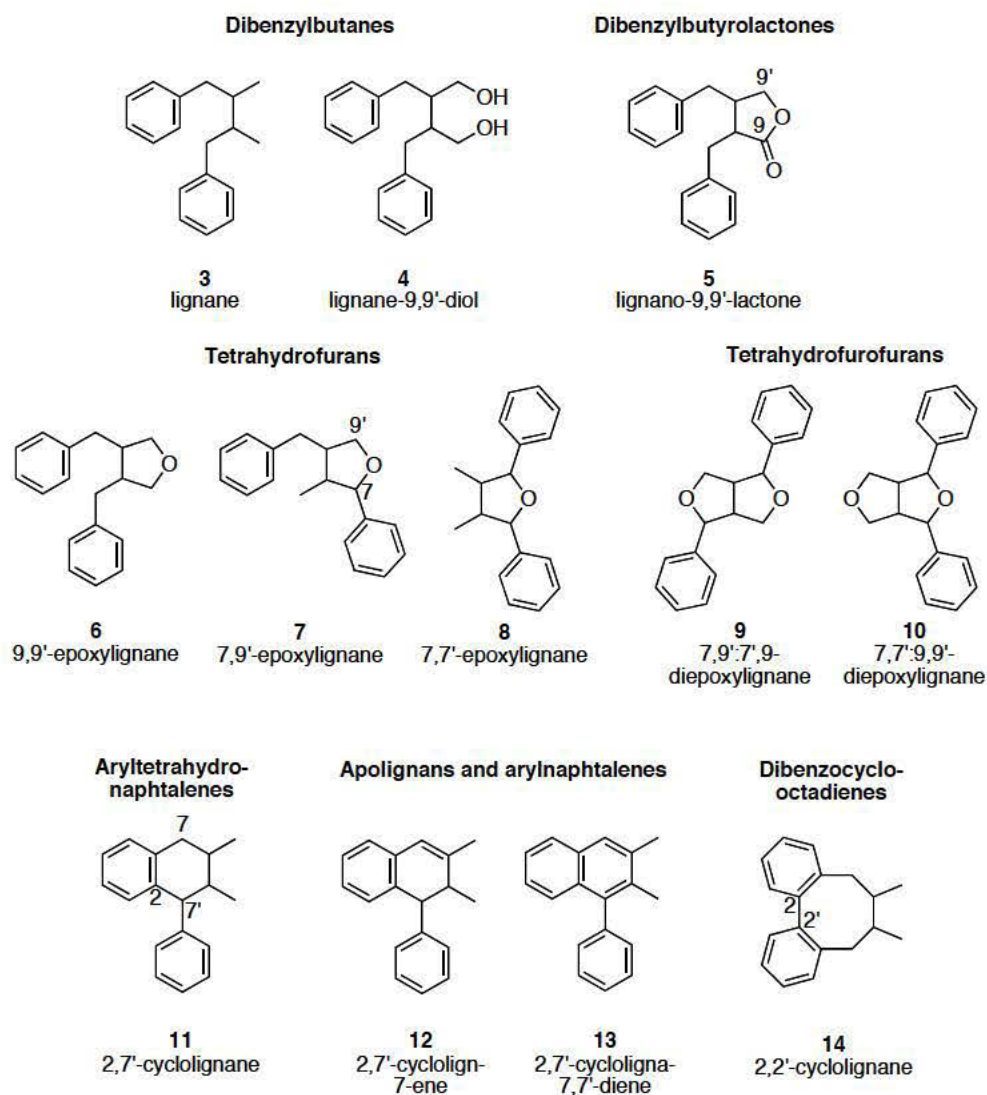


**Figure 3** Examples of structures related to lignans.

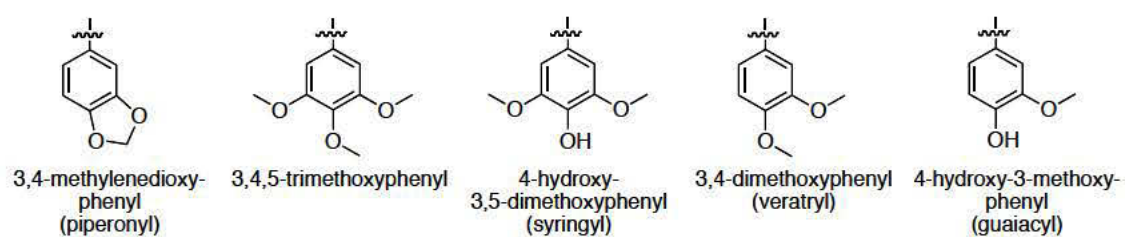
## 1.2 Classification

An early classification, based on the skeletal arrangements, was first introduced by Haworth in 1941.<sup>31</sup> He divided the lignans into three sub-groups: diarylbutanes, tetrahydronaphthalenes and tetrahydrofurans. Hearon and MacGregor expanded this first classification to five groups: 1,4-diarylbutanes **3–4**, 2,3-dibenzylbutyrolactones **5**, tetrahydrofurans **6–8**, tetrahydrofurofurans **9–10**, and 4-aryltetrahydronaphthalenes **11**.<sup>29</sup> This classification is still used today, with integration of two new groups, apolignan and aryl-naphthalene derivatives **12–13** and dibenzocyclo-octadienes **14**,<sup>15</sup> as presented in Figure 4.

Lignans of each subgroups may vary in oxidation levels of both the aromatic rings and propyl side chains. Due to their biosynthesis, all plant-originated lignans are *para*-substituted. The most frequently occurring aromatic rings found in naturally occurring lignans are presented in Figure 5. So far no plant lignan has been isolated with an unsubstituted phenyl ring.<sup>8</sup> However, an unsubstituted mammalian lignan, lignane-9,9'-diol **4** (2,3-dibenzylbutane-1,4-diol) is derived from human urine.<sup>32</sup>



**Figure 4** Classification of lignans, and the IUPAC nomenclature of the parent lignan skeleton.



**Figure 5** The most common aromatic substitution patterns in naturally occurring lignans.

### 1.3 Numbering and nomenclature

Over the years a variety of different numbering and nomenclature systems have been used for lignan compounds. At first, lignans were given trivial or semi-systematic names. With the increase of study and the number of isolated and synthetic structures, similar lignans have been named differently. Very similar names were given to different compounds, or one compound has been given several names. This has caused misunderstandings and mistakes. The IUPAC recommendations for the nomenclature of lignans and neolignans<sup>26</sup> brought back the first reasonable effort for a nomenclature system by Freudenberg and Weinges in 1961.<sup>30</sup> They suggested that the phenylpropane units should be numbered from 1–9 (the left half of the molecule) and 1'–9' (the right half of the molecule, as shown in **1**, Figure 2), where the 8–8'-linkage is assigned to all lignan classes according to the definition. However, the numbering depended on how the lignan was drawn, which made the scheme confusing. Ayres and Loike<sup>8</sup> amplified the system by proposing the use of unprimed locants for that half of the molecule which contained functionalities with priority according to IUPAC (structures **5**, **7** and **11** in Figure 4). In case of equivalent aliphatic chains, aromatic substituents are taken into account. In this thesis the IUPAC general alphabetical order of prefixes<sup>33</sup> is preferred instead of the system of prioritised aryl groups of Ayres and Loike.<sup>8</sup>

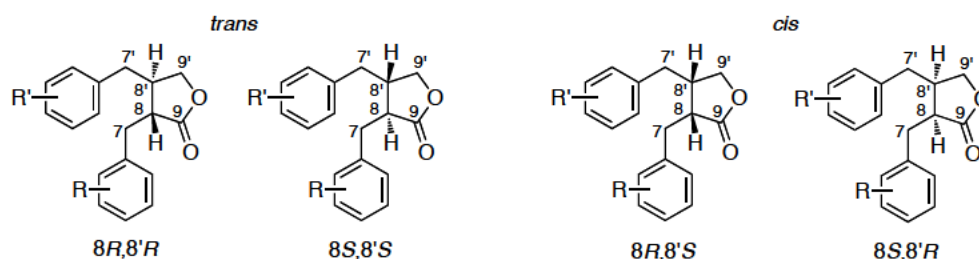
In the early review of natural resins by Haworth,<sup>25</sup> the original spelling was lignane, but the “e” was deleted in subsequent publications. However, the IUPAC recommendation on the nomenclature of lignans partly reinstated it: the class names lignan and neolignan are still spelled in the conventional way without a terminal “e”, but the parent structures below are spelled with a terminal “e” to indicate a saturated chain. The presence of a double or a triple bond in the side chain is indicated by changing the -ane ending to -ene (structure **12** in Figure 4) or -yne with the locant to indicate the position of the double or triple bond. If there are two double bonds then the -ane ending is changed to -diene with retention of the “a” (**13** in Figure 4).<sup>26</sup>

IUPAC recommendations for naming of different lignan skeletons are presented in Figure 4. Although a consistent nomenclature is now available, it takes time before it becomes generally accepted and permeates through the whole field. The trivial names of naturally occurring lignans are still commonly used in literature, and will be used also in this thesis, when they do not lead to misunderstanding.



## 2 Absolute configuration of lignans

Already in the early lignan research, it was found that lignans are optically active compounds, in practice all except the symmetrical ones, having chiral centers even in the simplest structures. For example each lignano-9,9'-lactone has four diastereomers due to the two chiral carbons C-8 and C-8'. The 8,8'-*trans* configuration is the most common, but also 8,8'-*cis* isomers are known.<sup>8</sup> In addition to the chiral carbons, there are prochiral centres (e.g. C-7, C-7', C-9', Figure 6).



**Figure 6** The diastereomers of lignano-9,9'-lactones.

The stereochemistry of lignans and the assignment of the absolute configuration are discussed in this section. In this thesis the focus is on the lignano-9,9'-lactone and 9'-hydroxylignano-9,7'-lactone structures.

### 2.1 Stereochemistry of lignans

In the early lignan research, the optical activity was indicated using trivial names with prefixes *d* and *l* or (+) and (–) to show the direction of the rotation of plane-polarised light. Like the various naming and numbering systems (Chapter 1.3), also these signs can be misleading. The angle by which the sample rotates the plane-polarised light, is experimental information, dependent on several factors, e.g. concentration and temperature. The sign of rotation of polarised light can even change in different solvents (for example both epimers of 7'-hydroxymatairesinol are levorotatory in THF solvent, but in EtOH the value of optical rotation is positive for the (7'*R*)-epimer and negative for the (7'*S*)-epimer).<sup>34,35</sup> Also, it does not reveal the absolute stereochemistry, the fundamental three-dimensional architecture of the molecule, nor the enantiomeric purity of lignans (naturally occurring or synthetic).

The stereochemistry of the two chiral centres C-8 and C-8' was initially assigned by derivatisation or chemical conversion into stereochemically known molecules and comparison with analogous structures.<sup>29,36,37</sup> Nowadays, in addition to derivatisation, total syntheses and advanced analytical techniques, such as NMR, X-ray spectroscopy<sup>38</sup> and chiroptical methods,<sup>39</sup> are used to

resolve the structural and stereochemical problems. However, despite the advanced spectroscopic techniques, the process of structure assignment is still a tricky task in many cases, even with relatively simple structures like lignans, and mistakes are a relatively common occurrence.<sup>40,41</sup>

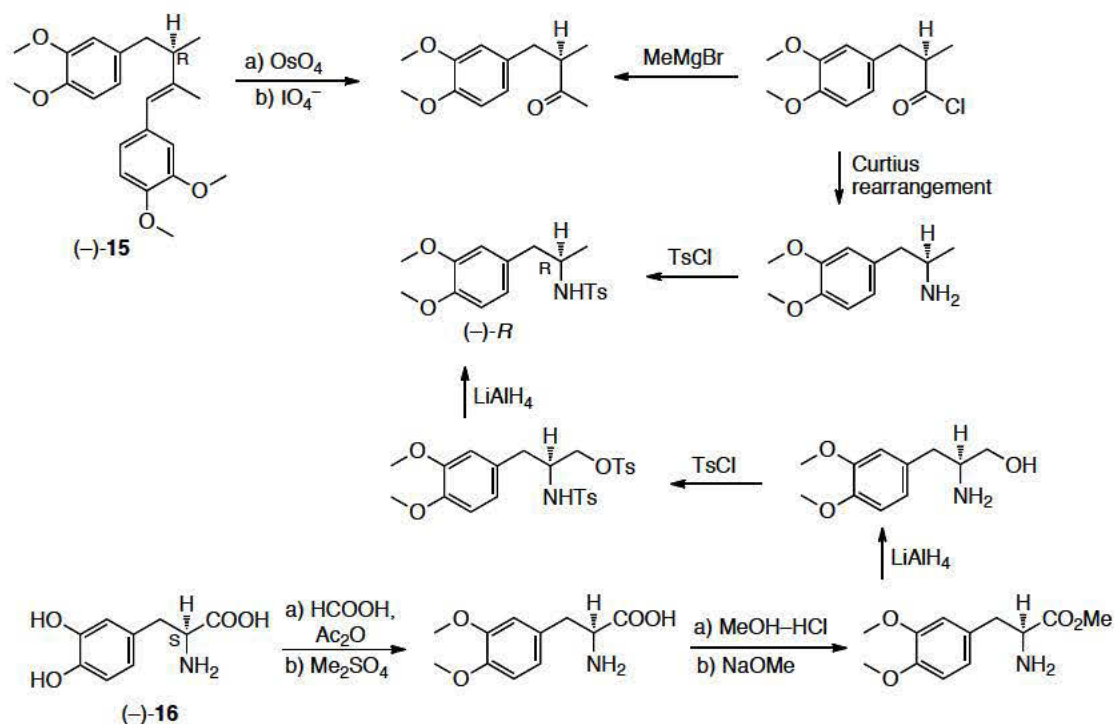
In the early nomenclature system of Freudenberg and Weinges, it was proposed that the stereochemistry could be expressed with the description  $\alpha$ , when hydrogen atom is pointing up from the plane, and  $\beta$ , when hydrogen is pointing down.<sup>30</sup> Unfortunately, this symbolism is prone to errors, since it requires the molecules to be drawn according to specific rules. To avoid misunderstandings, Ayres and Loike recommended the use of the unambiguous Cahn–Ingold–Prelog *R/S* system<sup>42,43</sup> to express the absolute configurations,<sup>8</sup> and it is preferred also in this thesis and in the original articles, even if the IUPAC recommendations brought back the signs  $\alpha$  and  $\beta$ , which again has certain demands on how the molecule must be drawn.<sup>26</sup> The IUPAC recommendations in using descriptions  $\alpha$  and  $\beta$  are different from the early nomenclature system of Freudenberg and Weinges: now the descriptors  $\alpha$  and  $\beta$  refer to the location of a bridgehead or ring hydrogen or substituent below or above the plane of the molecules, respectively. As also the drawing instructions are different between these two systems, the stereochemistry descriptors for a certain chiral carbon in e.g. a lignano-9,9'-lactone (**5**) structure may differ depending on which nomenclature system is used.

## 2.2 Stereochemistry at carbons 8 and 8'

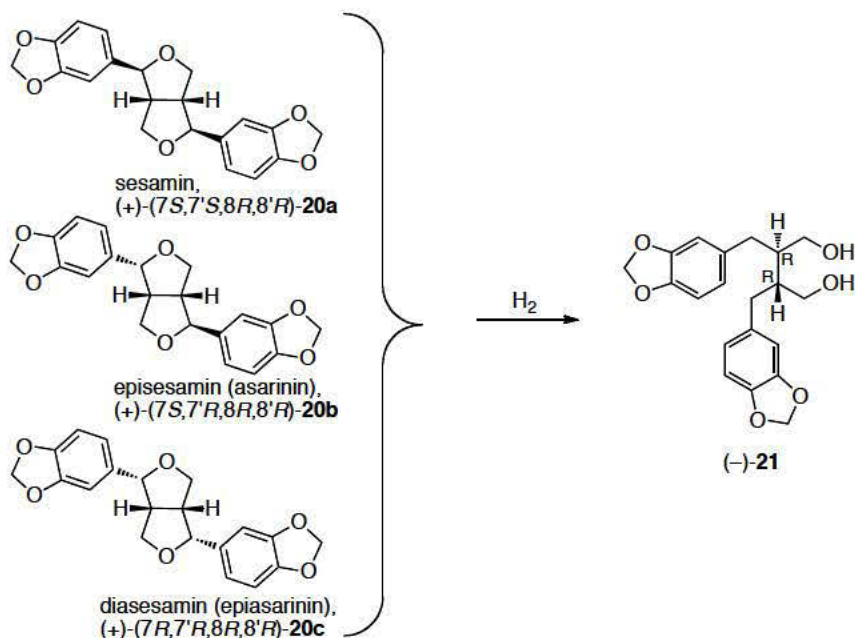
The proposed 8,8'-*trans* configuration of natural lignano-9,9'-lactones (–)-matairesinol and (–)-hinokinin<sup>44</sup> was initially confirmed by reduction with  $\text{LiAlH}_4$  to optically active diols.<sup>45</sup> If the compounds had possessed the *cis* configuration, the reduction would have led to optically inactive *meso*-products.

Dimethyl guaiaretic acid (–)-**15** was the first lignan whose absolute configuration was determined in 1957. The absolute configuration of the sole chiral carbon was determined by Schrecker and Hartwell by relating it chemically to L-(*S*)-3,4-dihydroxyphenylalanine (–)-**16** (Scheme 1).<sup>37</sup> Two years earlier they had demonstrated that matairesinol (–)-**17**, secoisolariciresinol (–)-**18** and dihydroguaiaretic acid (–)-**19** related to the absolute configuration of dimethyl guaiaretic acid (–)-**15**.<sup>46</sup>

The absolute configurations of the bridge carbons (8 and 8') of furofuran lignans were resolved through conversions: catalytic hydrogenation of the three dextrorotatory diastereomers (+)-**20a–c** led to levorotatory dihydrocubebin (–)-**21** (Scheme 2),<sup>47</sup> which had the same absolute configuration as secoisolariciresinol (–)-**18**.<sup>48</sup> In Scheme 3 the correlation between optical rotation, absolute configuration and various lignan skeletal oxidation levels of the guaiacyl substituted (8*R*,8'*R*)-lignans are shown.

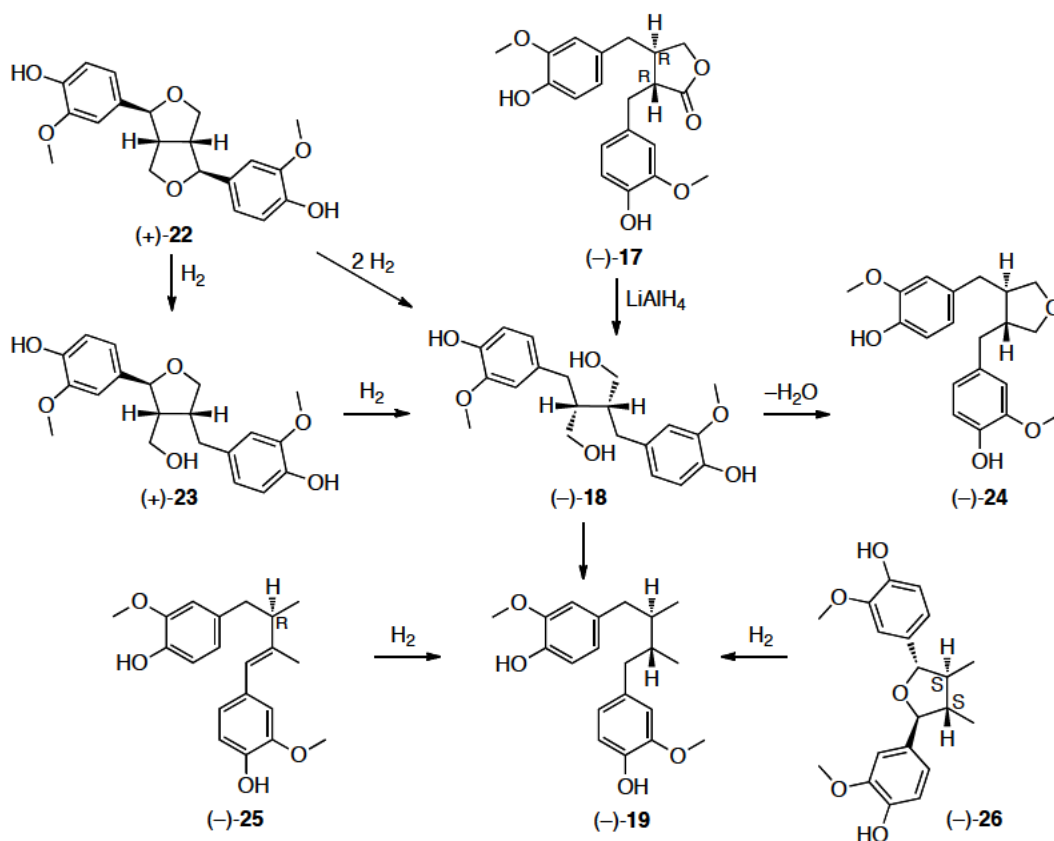


**Scheme 1** Conversion of (-)-dimethyl guaiaretic acid **15** into L-3,4-dihydroxyphenylalanine (-)-**16**, a natural aminoacid, whose absolute stereochemistry was known. The key step is the Curtius rearrangement of acylazide, which retains the configuration.<sup>37</sup>



**Scheme 2** Catalytic hydrogenation of the three dextrorotatory diastereomers of piperonyl substituted tetrahydrofuran lignans (+)-**20a–c** lead to levorotatory dihydrocubebin (-)-**21**,<sup>47</sup> which has the same absolute configuration as secoisolariciresinol (-)-**18**.<sup>48</sup>





**Scheme 3** The correlation between optical rotation and various lignan skeletal oxidation levels of the guaiacyl substituted lignans that have the absolute configuration of  $(8R,8'R)$ .<sup>49</sup> Note that the *S* configuration at 8 and 8' in the (-)-frangasin  $\text{A}_2$  26 corresponds to the *R* configuration in all the other structures.

Big efforts were necessary in establishing the absolute configurations of lignans in the early stage of lignan research, when modern, advanced analytical techniques, such as NMR or X-ray spectroscopy, were not available or routine like now, and all the determinations had to be done via chemical conversions into known molecules. Despite the gathered early knowledge of correlation between optical rotations and absolute configurations of given lignan skeletons, there are inaccuracies, misunderstandings and even errors in the literature, which have caused – and are still causing – some inconsistencies. In many cases only relative configurations have been determined, and signs of optical rotation are connected to wrong enantiomers, i.e. stereoisomers that are mirror images to each other.

An example of an erroneous interpretation that has survived for a long time in the literature is the case of frangasin  $\text{A}_2$  (-)-26 (Scheme 3). In a review of lignans and cyclo lignans in 1967,<sup>49</sup> Weinges and Spänig deduced (from the prevailing structural and stereochemical knowledge) the absolute configuration and signs of optical rotation for some compounds before they were actually been synthesised or found in the nature. Frangasin  $\text{A}_2$  (-)-26 (at that time Weinges and Spänig called it dimethylether-(-)-galbelgin) is a guaiacyl substituted lignan stereochemically related to the others in Scheme 3, and was

inferred to be levorotatory.<sup>49</sup> The absolute configuration of the corresponding veratryl substituted analog (–)-galbelgin **27** was elucidated by Birch *et al.* in 1958.<sup>50</sup> Eventually, when the structure of fragransin A<sub>2</sub> **26** was later isolated (from species *Myristica fragrans*) in 1987,<sup>51</sup> the isolated lignan was the (+)-enantiomer, but it was erroneously linked to the stereochemistry of (–)-galbelgin.<sup>50</sup> The wrong stereochemical assignment<sup>51,52</sup> was not corrected until 2007 by enantioselective synthesis,<sup>53</sup> but for example Reaxys, the online version of Beilstein Crossfire database, still gives the wrong stereochemical information in search. The confusions are problematic, since an occasional reader or a newcomer in the lignan field is easily led astray.

A correlation between physical data (optical rotation and NMR data) and the absolute stereochemistry is observed. Thus, when the relative stereochemistry (*cis* or *trans*) at carbons 8 and 8' is determined, e.g. with the help of NOESY correlation, the absolute configuration may be assigned, if the sign of the optical rotation is known. For instance levorotatory (–)-enantiomers of the 8,8'-*trans*-dibenzylbutane-9,9'-diols, 8,8'-*trans*-9,9'-epoxylignanes, and 8,8'-*trans*-lignano-9,9'-lactones have been shown to possess the same absolute *R,R* configuration at C-8 and C-8' with respect to carbon skeleton,<sup>54,55</sup> while the (8*S*,8'*S*)-isomers are dextrorotatory.<sup>55,56,57</sup> The less common *cis*-structures (8*S*,8'*R*)-lignano-9,9'-lactones are dextrorotatory,<sup>58,59,60</sup> whereas the (8*R*,8'*S*)-lignano-9,9'-lactones are levorotatory.<sup>60,61,62\*</sup> When more chiral carbons are involved, the comparison becomes more complicated but nonetheless is possible.

## 2.3 Stereochemistry at carbons 7 and 7'

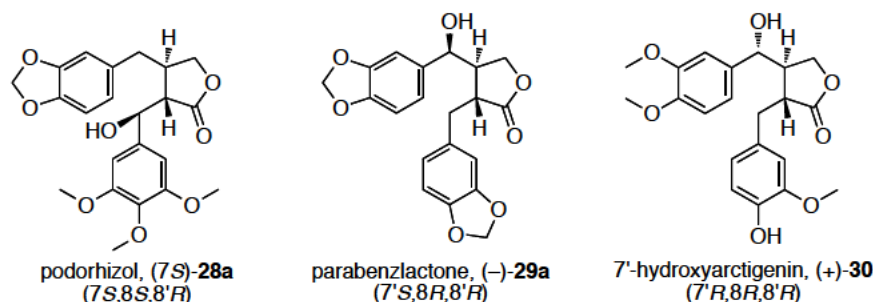
For natural lignans, the substituents at carbons 7 and 7' are hydroxy or alkoxy groups. The stereochemistry at C-7 for naturally occurring 7-hydroxylignano-9,9'-lactone podorhizol (7*S*)-**28a** was established as *S* already in 1967. The configuration was deduced from the existence of a hydrogen bond between the hydroxyl and the lactone carbonyl, possible for one enantiomer only (Figure 7).<sup>63</sup> However, the stereochemistry at C-7' has been uncertain until 2002 owing to conflicting literature data.

The plant lignan, 7'-hydroxylignano-9,9'-lactone parabenzlactone (–)-**29a** was isolated for the first time in 1970.<sup>64</sup> In 1976 its stereochemistry was assigned (erroneously) as (7'*R*),<sup>65</sup> because the deduction was based on the absolute configurations of podorhizol (7*S*)-**28a** and its epimer epipodorhizol (7*R*)-**28b**, determined by Kuhn and Wartburg.<sup>63</sup> The <sup>1</sup>H-NMR parameters of H-7 ( $\delta$  and *J*) of podorhizol (7*S*)-**28a** and epipodorhizol (7*R*)-**28b** were applied<sup>65,66</sup> to show that (–)-parabenzlactone possess the (7'*R*) configuration. Obviously the

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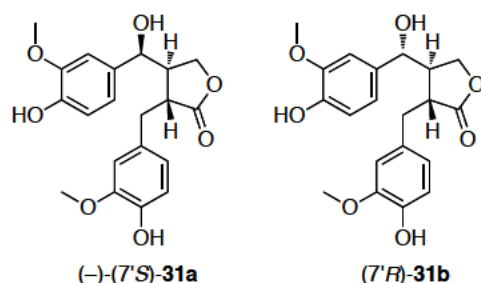
\* In the ref. 62 the stereodescriptors (8*S*,8'*R* and 8*R*,8'*S*) and the optical rotations are probably connected wrong way round, since they are in conflict with the other reported data,<sup>58–61</sup> including the work of Ward *et al.*<sup>59</sup> which they refer to.

analysis is fundamentally prone to errors, since the environment of the 7- and 7'-sites are different (Figure 7).



**Figure 7** Podorhizol (7*S*)-**28a**, (-)-parabenzlactone **29a** and (+)-hydroxyarctigenin **30**. (Note that the *S* configuration at C-8 in the 7-OH-lignano-9,9'-lactone corresponds to the *R* configuration in 7'-OH-lignano-9,9'-lactone. Thus in the case of podorhizol (8*S*,8'*R*) corresponds to the *trans* configuration.)

(7'*S*)-Hydroxymatairesinol (7'*S*)-**31a** and its epimer (7'*R*)-**31b** were first isolated in 1957 by Freudenberg and Knof,<sup>34</sup> but the absolute stereochemistry at C-7' was uncertain for many decades. Since 7'-hydroxymatairesinol **31** is amorphous, no crystal structure was available and exhaustive NMR studies were carried out with differing results.<sup>66,67,68</sup> The absolute stereochemistry of the two benzylic epimers (7'*R*) and (7'*S*) of **31** (Figure 8) was clarified only in 2002 by Eklund *et al.*, who converted the (7'*S*) isomer **31a** into a crystalline triol lignan which could be analysed by X-ray crystallography.<sup>35</sup>



**Figure 8** The epimers of hydroxymatairesinol **31**. The sign of optical rotation for the (7'*R*) epimer **31b** depends on the solvent used. In THF it is levorotatory, but in ethanol dextrorotatory.<sup>34,35</sup>

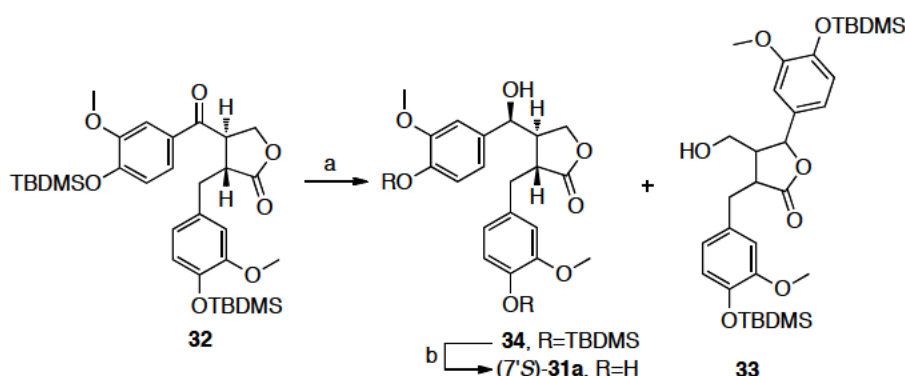
The true absolute configuration of the natural 7'-hydroxylignano-9,9'-lactone (+)-(7'*R*)-hydroxyarctigenin **30** (Figure 7) was established by Fisher *et al.* by means of total asymmetric synthesis.<sup>69</sup> In our laboratory Raffaelli *et al.* have worked on a general approach for the stereoselective synthesis of several (7'*S*,8*R*,8'*R*)-hydroxylignano-9,9'-lactones,<sup>70</sup> and one of the target molecules was (7'*S*)-parabenzlactone **29a**. The physical and spectroscopical data obtained for synthetic (7'*S*)-parabenzlactone were in good agreement with those reported for the natural product (-)-**29a**<sup>64,65</sup> confirming that the

stereochemistry of (–)-parabenzlactone **29a** had been erroneously assigned as (7'*R*) instead of (7'*S*).

NMR data can be used to determine the relative configuration of 7'-hydroxylignano-9,9'-lactones. When the relative stereochemistry at carbons 8 and 8' is assigned by a NOESY experiment, the 7'-epimers are separable from each other by comparing the signal for 7'-protons. When deuterated chloroform is used as a solvent, the 7'-H for the *S* isomer (7'*S* relative to 8*R*,8'*R*') appears at about  $\delta$  4.6 ppm<sup>69,70,71,72</sup> and for the *R* isomer (7'*R* relative to 8*R*,8'*R*') at about  $\delta$  4.4 ppm, irrespective of the aromatic substituents (OH, OMe, OTBDMS, OCH<sub>2</sub>O).<sup>69,73</sup> When deuterated acetone is used as a solvent, both signals shift slightly downfield.<sup>70,73,74,75</sup>

## 2.4 Stereochemistry of 9'-hydroxylignano-9,7'-lactones

In our laboratory Raffaelli *et al.* studied the stereoselective synthesis of (7'*S*,8*R*,8'*R*')-hydroxylignano-9,9'-lactones.<sup>70</sup> In the final step, a stereoselective reduction of the 7' keto group with L-Selectride, an unexpected byproduct was obtained in addition to the expected (7'*S*)-OH compound (Scheme 4). The byproduct from the reaction of 7'-oxolignano-9,9'-lactone **32** was isolated in about 10 % yield. Spectroscopic data (IR, 1- and 2-D NMR, MS) suggested that the compound was a rearranged hydroxylactone **33**.

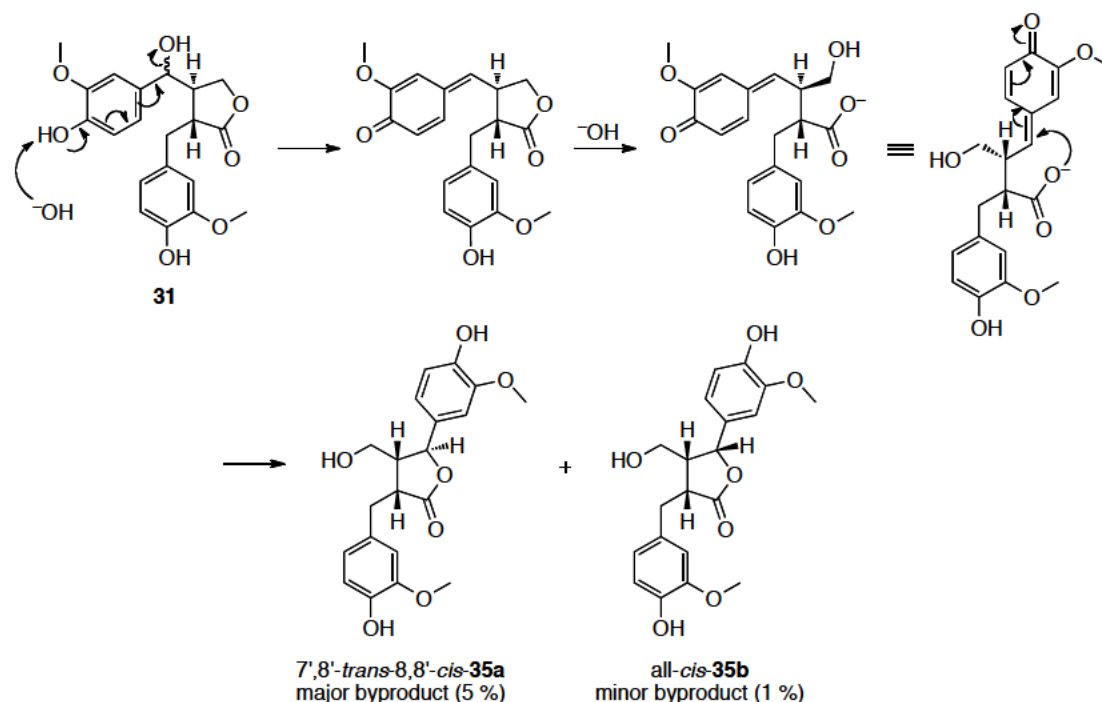


**Scheme 4** L-Selectride reduction of a 7'-oxolignano-9,9'-lactone **32**.<sup>70</sup> Reagents and conditions: a) L-Selectride, THF, –78 °C; b) TBAF–CH<sub>3</sub>COOH, THF, 0 °C.

The L-Selectride reaction was carried out for several 7'-oxolignano-9,9'-lactones, with various aromatic substituents.<sup>70</sup> The byproducts, even if not isolated, were easily observed in the <sup>1</sup>H-NMR spectrum of the crude product, since they had a proton signal (a doublet at about  $\delta$  5.4 ppm, for **33**  $J = 2.6$  Hz) in a region with no other signals.

At about the same time Eklund *et al.* reported the isolation and characterisation of two epimeric lariciresinol type lactone lignans isohydroxymatairesinol **35a** and epi-isohydroxymatairesinol **35b** from the treatment of 7'-hydroxymatairesinol **31** with base (Scheme 5).<sup>76</sup> The

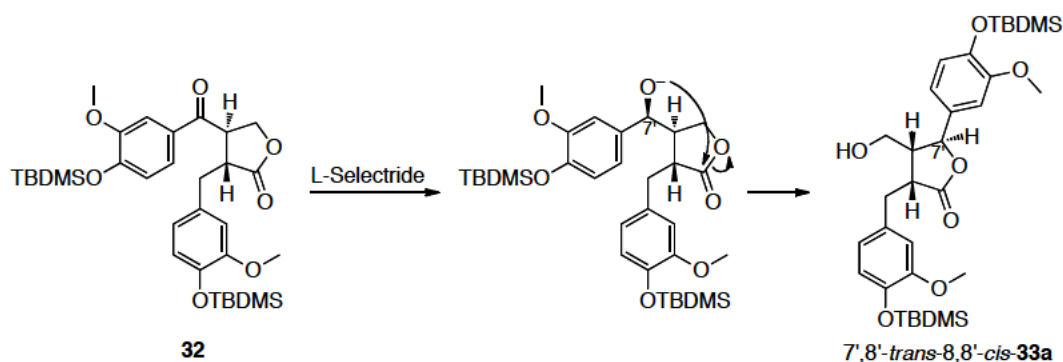
stereochemistry of the products **35a** and **35b** were deduced from the coupling constants and NOESY experiments. The NMR data (H-7',  $\delta$  5.46 ppm, d,  $J$  = 2.6 Hz) suggested that the major byproduct of Eklund *et al.* (7',8'-*trans*-8,8'-*cis*-**35a** in Scheme 5) and the rearranged byproducts of Raffaelli *et al.* (e.g. **33** in Scheme 4) possessed the same skeleton (differing only in the aromatic substituents) and 7',8'-*trans*-8,8'-*cis*-stereochemistry.



**Scheme 5** Formation of isohydroxymatairesinol 7',8'-*trans*-8,8'-*cis*-**35a** and epi-isohydroxymatairesinol all-*cis*-**35b** from hydroxymatairesinol **31** via a *para*-quinone methide intermediate in aqueous alkaline conditions.<sup>76</sup>

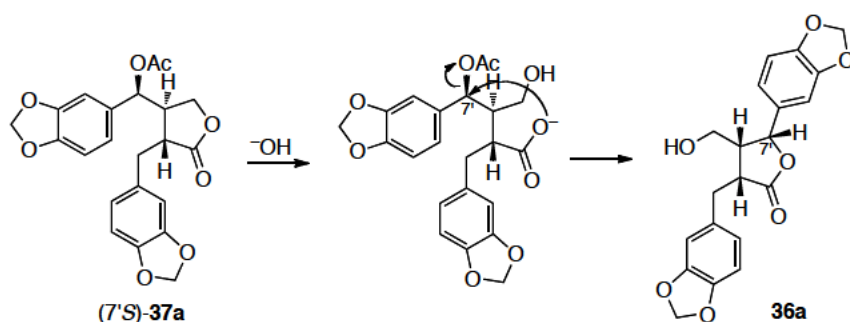
Eklund *et al.* proposed that their lactones were formed via a *para*-quinone methide mechanism (Scheme 5), accessible when a free *p*-phenol moiety is present. However, in the case of the reactions of Raffaelli *et al.* the *p*-phenols were protected. Additionally, no further rearranged lactones were detectable in the NMR of the crude mixture from the treatment of 7'-oxolignano-9,9'-lactones with L-Selectride, contrary to Eklund *et al.* who obtained two epimeric byproducts (ratio between isomers 5:1), but that could result from the much smaller reaction scale: Raffaelli's *et al.* reduction reactions were done with some dozen milligrams while Eklund *et al.* were operating with over ten gram scale of hydroxymatairesinol isolated from Norway spruce knots. In any case, the quinone methide mechanism was ruled out in the case of Raffaelli *et al.* and translactonisation (with retention of the configuration at all chiral carbons) was hypothesised instead (Scheme 6).





**Scheme 6** Mechanism of translactonisation.<sup>70</sup>

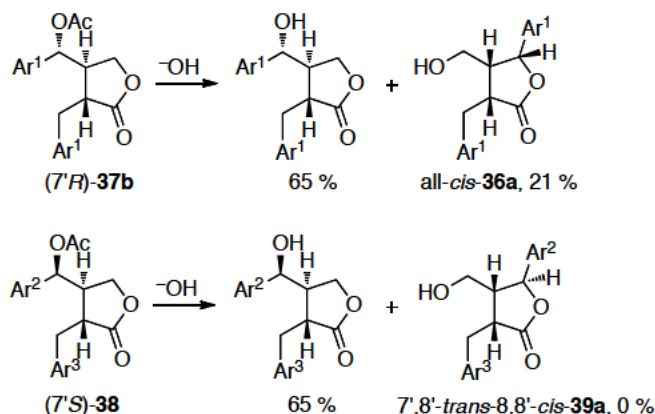
In the literature there were few other examples of formation of rearranged hydroxylactones similar to compounds **33a**, **35a** and **35b**. Niwa *et al.*<sup>65</sup> reported the formation of a rearranged lactone **36a** as a side product from the treatment of natural acetylparabenzlactone **37** with KOH (Scheme 7). They suggested an all-*cis* structure for this compound. According to <sup>1</sup>H NMR (H-7',  $\delta$  5.13 ppm, d,  $J$  = 8.0 Hz) it seemed analogous with all-*cis*-**35b**, the minor byproduct of Eklund *et al.*<sup>76</sup> However, the absolute configuration of the natural (–)-parabenzlactone (**29a**) was shown to be (7'*S*) (and not (7'*R*) as Niwa *et al.* had proposed, as discussed earlier in the Chapter 2.3), and therefore the mechanism of the formation in the case of **36a** could not be similar translactonisation (attack by 7'-O<sup>–</sup> on the lactone ring) presumed for **33a** (Scheme 6). Thus S<sub>N</sub>2 mechanism was considered:<sup>70</sup> the lactone undergoes hydrolysis, then the generated COO<sup>–</sup> acts as a nucleophile and attacks the 7' carbon *via* S<sub>N</sub>2 mechanism, detaching the AcO group and resulting a new lactone ring with inversion of the configuration at carbon 7' (Scheme 7).



**Scheme 7** Proposed mechanism<sup>70</sup> for the formation of Niwa's *et al.*<sup>65</sup> rearranged all-*cis*-lactone.

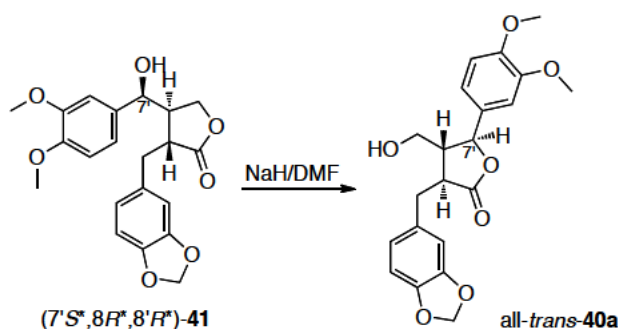
Furthermore, the same group later reported that on treatment of 7'-acetylhydroxyarctigenin monoacetate **38**, which at that time was believed to be (7'*S*,8*R*,8'*R*), under similar basic conditions, no rearrangement occurred (Scheme 8).<sup>66</sup> No explanation was given for this behavior, even it could be expected that the rearrangement from a compound (7'*S*)-**38** via a simple

translactonisation would take place more easily than from (7'*R*)-**37b**, since the rearranged product 7',8'-*trans*-8,8'-*cis*-**39a** may be considered sterically more favorable than the all-*cis*-**36a** (Scheme 8). However, as discussed above and earlier in the Chapter 2.3, the stereochemistry of the starting materials were incorrect (the parabenzlactone (–)-**29a** was erroneously assigned as 7'*R*), and in the Chapter 6.3.1.3 it will be argued that the configuration of the product **36a** is incorrect here as well.



**Scheme 8** Reported saponification of acetylparabenzlactone **37b**<sup>65</sup> and acetylhydroxyarctigenin monoacetate **38**.<sup>66</sup> Ar<sup>1</sup> = piperonyl, Ar<sup>2</sup> = 3,4-dimethoxyphenyl, Ar<sup>3</sup> = 4-acetyloxy,3-methoxyphenyl.

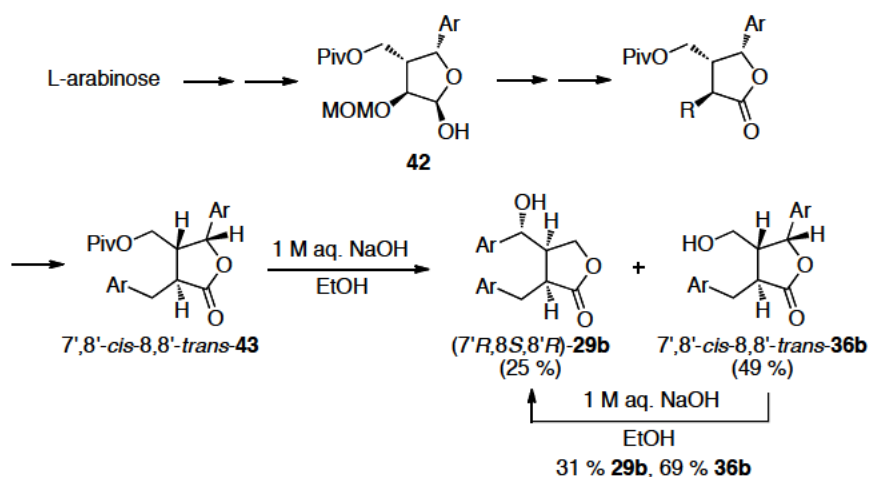
The other example was from Moritani *et al.*, who obtained the rearranged lactone **40a** by treating racemic 7'-hydroxylignano-9,9'-lactone **41** with NaH in DMF (Scheme 9).<sup>71</sup> Their product had a proton signal at  $\delta$  5.16 ppm (H-7', d,  $J$  = 9.2 Hz). The structure of the rearranged product **40a** was unambiguously determined as all-*trans* by X-ray crystallographic analysis, and showed that epimerisation had occurred at the carbonyl  $\alpha$  carbon during the rearrangement.



**Scheme 9** Rearrangement of (7'*S*\*,8*R*\*,8'*R*\*)-7'-hydroxylignano-9,9'-lactone (7'*S*\*,8*R*\*,8'*R*\*)-**73** in NaH/DMF affording all-*trans*-**40a** as the only product.<sup>71</sup>

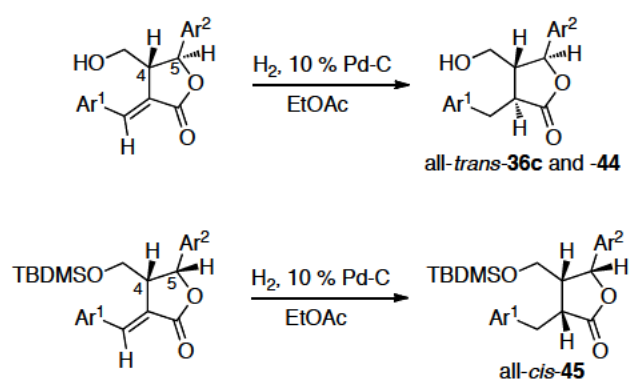
Yamauchi *et al.* used the translactonisation reaction to access *cis*-8,8'-lignano-9,9'-lactone<sup>60,77</sup> and *cis*-8,8'-aryltetrahydronaphthalene<sup>54</sup> structures (Scheme 10). They synthesised a chiral hemiacetal **42** from L-arabinose. The hemiacetal

was oxidised and stereoselectively  $\alpha$ -benzylated to afford a pivaloyl protected 9'-hydroxylignano-9,7'-lactone (7',8'-*cis*-8,8'-*trans*-**43**). The targeted 8,8'-*cis*-7'-hydroxylignano-9,9'-lactone (7'*R*,8*S*,8'*R*)-**29b** was obtained via translactonisation (no epimerisation). Treatment of **43** with aq. NaOH/EtOH yielded (7'*R*,8*S*,8'*R*)-**29b** and the deprotected 9'-hydroxylignano-9,7'-lactone 7',8'-*cis*-8,8'-*trans*-**36b** in a ratio of about 1:2.<sup>77</sup>



**Scheme 10** Rearrangement of pivaloyl protected 7',8'-*cis*-8,8'-*trans*-9'-hydroxylignano-9,7'-lactone **43**. Treatment in aq. NaOH/EtOH yields a 2:1 mixture of **36b** and **29b**.<sup>77</sup> Ar = piperonyl.

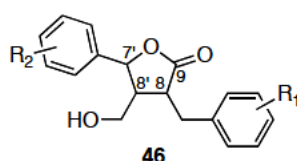
In addition to the rearranged 9'-hydroxylignano-9,7'-lactones above, there is another synthetic example in the literature. During a stereospecific synthesis of dihydrosesamin, Stevens and Whiting studied the stereochemistry of additions to 3-arylidine lactones and reported 9'-hydroxylignano-9,7'-lactones as intermediates in both all-*trans*- and all-*cis*-configurations (Scheme 11).<sup>78</sup>



**Scheme 11** All-*trans*- and all-*cis*-9'-hydroxylignano-9,7'-lactones from the hydrogenation of 3-arylidine lactones. The stereochemistry of additions to the 3-arylidine lactones is controlled by the 5-substituent rather than the 4-substituent.<sup>78</sup> **36c** and **45**: Ar<sup>1</sup> = Ar<sup>2</sup> = piperonyl, **44**: Ar<sup>1</sup> = phenyl, Ar<sup>2</sup> = piperonyl.



All the synthetic 9'-hydroxylignano-9,7'-lactone structures reported to date (also from the publications I and II) are listed in Table A1 (in Appendix, pp. 94–96). The 9'-hydroxylignano-9,7'-lactone structure **46** is not just a synthetic peculiarity, but it is also found in nature, though it is not included in any classification of lignans yet (see Figure 4, p. 12). The two examples found in nature to date are isohydroxymatairesinol **35a** and a sesquineolignan. Isohydroxymatairesinol (**35a**, Scheme 5, p. 21) is found in spruce knotwood,<sup>76,79</sup> sesame seeds<sup>80,81</sup> and cereals (e.g. rye and wheat),<sup>81</sup> and it has been detected in rat urine after hydroxymatairesinol (**31**, Figure 8, p. 19) administration.<sup>82</sup> The sesquineolignan having a hydroxylignano-9,7'-lactone structure has been isolated from heartwood of the Chinese conifer species *Tsuga dumosa*.<sup>83</sup>



As a summary, 7'-hydroxylignano-9,9'-lactones and 9'-hydroxylignano-9,7'-lactones can rearrange into each other under various basic hydrolytic conditions, the result of the reaction depending on the starting material and the reaction conditions. However, at the time of the paper of Raffaelli *et al.*<sup>70</sup> the reported rearrangements left confusion about the true stereochemistry of the products and the mechanism of formation. Different mechanisms were presented: *para*-quinone methide intermediate with racemisation at C-7' (and retention of the configuration at C-8);<sup>76</sup> S<sub>N</sub>2 mechanism with inversion of configuration at C-7';<sup>65,70</sup> translactonisation from NaH treatment with retention of the configuration at C-7' and concurrent α epimerisation at C-8;<sup>71</sup> and translactonisation with retention of the configuration at C-7' and C-8 from L-Selectride reduction<sup>70</sup> or treatment with NaOH in EtOH.<sup>54,60,77</sup> Our contribution on this topic is discussed in the Chapter 6.3 of this thesis.<sup>I,II</sup> We have revealed a number of stereochemical errors in the published literature, which were corrected, and have clarified some mechanistical aspects.

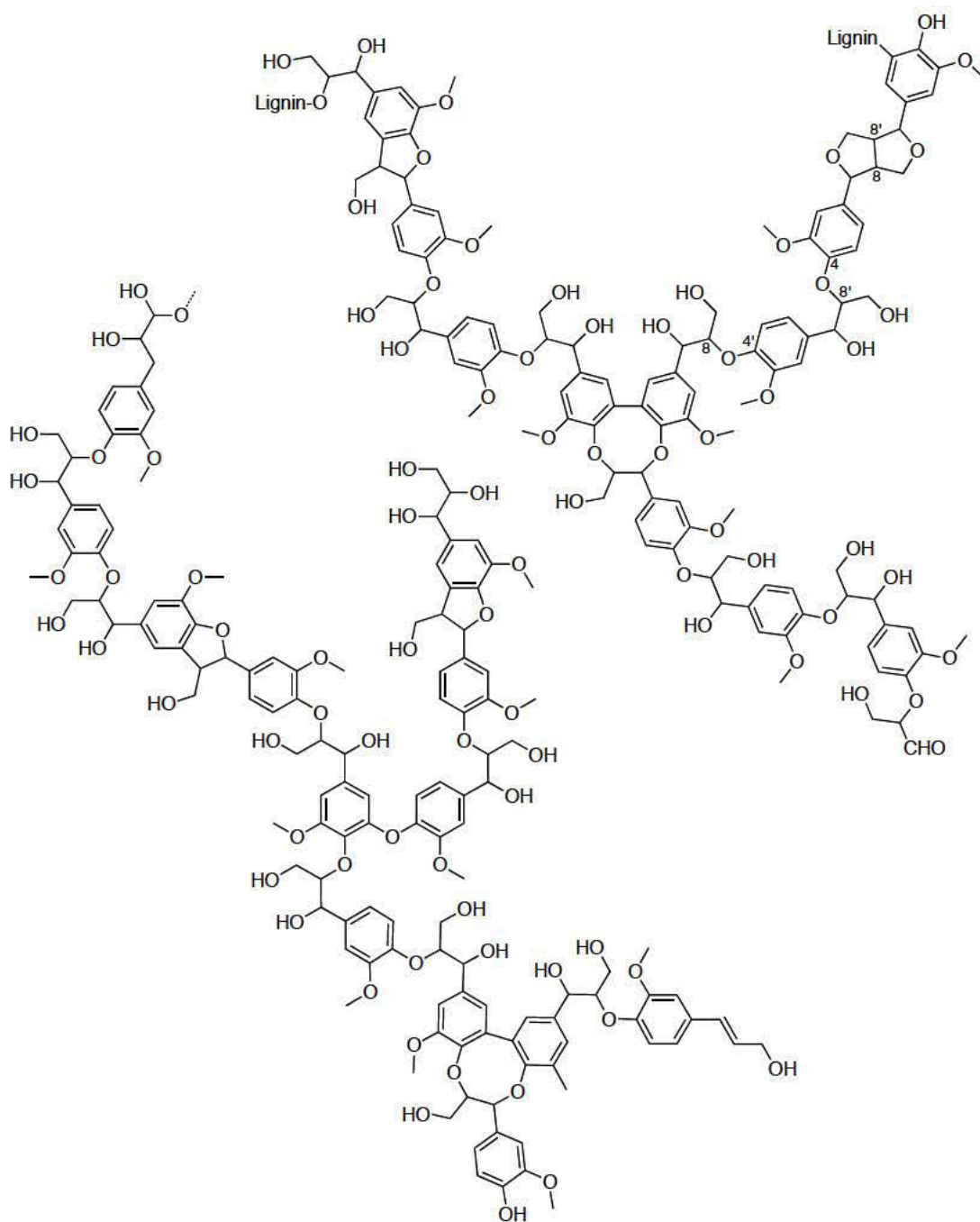
## 3 Biological properties of lignans

### 3.1 Lignan biosynthesis

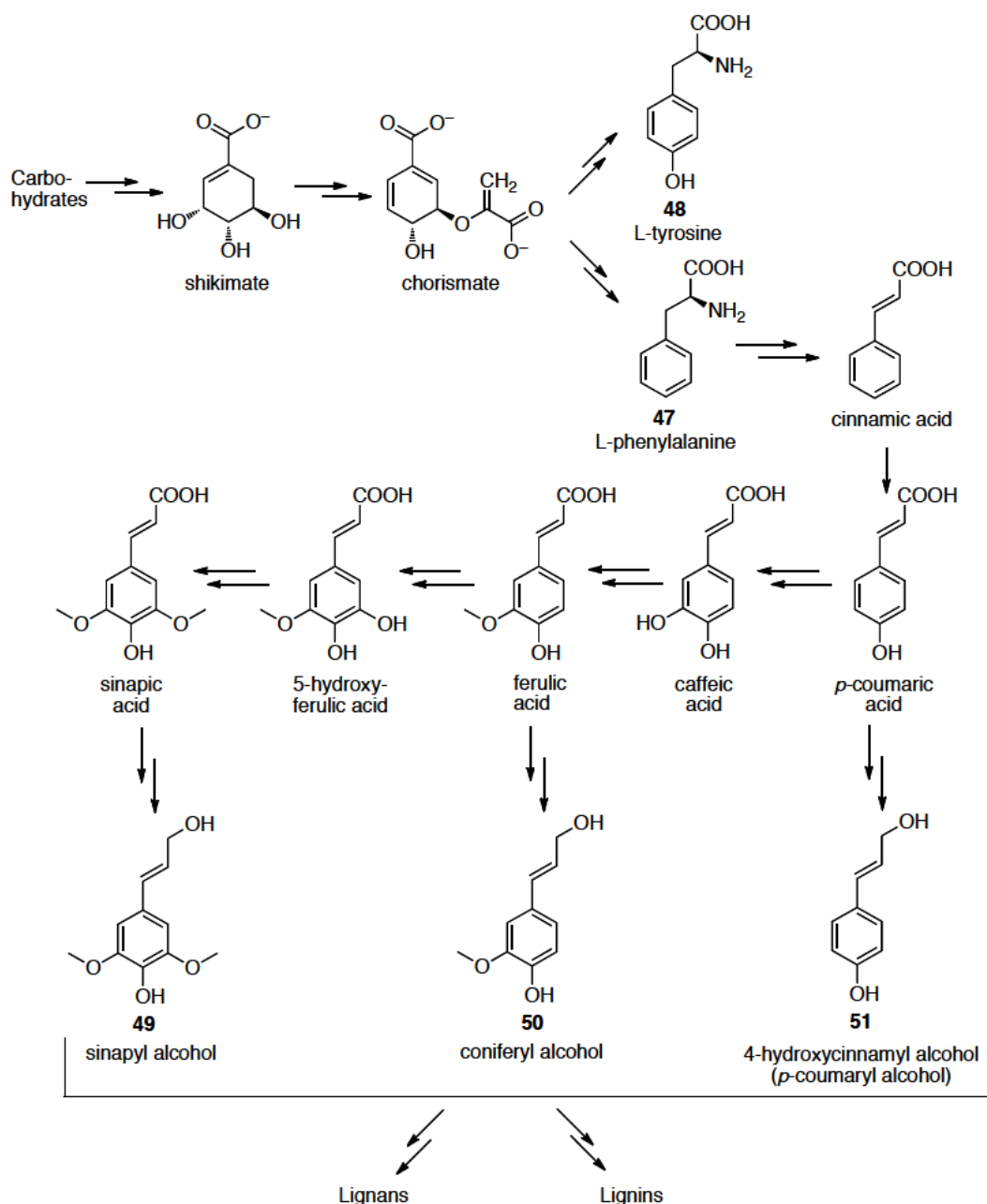
Biosynthesis of lignans is closely related to but yet distinct from those of other phenylpropanoids, such as norlignans, neolignans (Figure 3, p. 11) and lignin, which is a complex aromatic biopolymer (Figure 9).<sup>11,14</sup> Despite the biosynthesis of lignin and lignan are separate, in the literature they often are related because they are made up of the same starting materials, i.e. the same monomeric precursors are used in both lignin and lignan formation.

Lignans, together with lignins, are the major metabolic products of phenylpropanoid metabolism in vascular plants. They are derived from the shikimate-chorismate pathway, which also produces the aromatic amino acids, phenylalanine **47** and tyrosine **48** (Scheme 12). Extension of the phenylpropanoid pathway in vascular plants, from phenylalanine onwards, ultimately leads to both the dimeric and oligomeric lignans and to the polymeric lignin. The most important function of lignin is strengthening the cell wall of plants. It also provides the vasculature for water conduction. Lignans are the abundant group of closely related non-structural phenolic metabolites, which role in the plants is not yet completely understood, but it seems evident that the primary role of the lignans is in plant defense.<sup>12,84</sup>

Despite their seeming structural similarity and a close relationship between the evolution of lignan biosynthesis and of the other phenylpropanoid compounds (lignins and phenylpropanoid monomers), the lignin and lignan pathways are independent.<sup>12</sup> There is evidence to suggest that the lignan biosynthetic system was acquired prior to that of the lignin. Although there are no reports about lignans in algae, lignans have been found in “primitive” early land plants, such as liverworts (non-vascular bryophyte land plants), which do not contain lignin.<sup>11,12,85</sup>

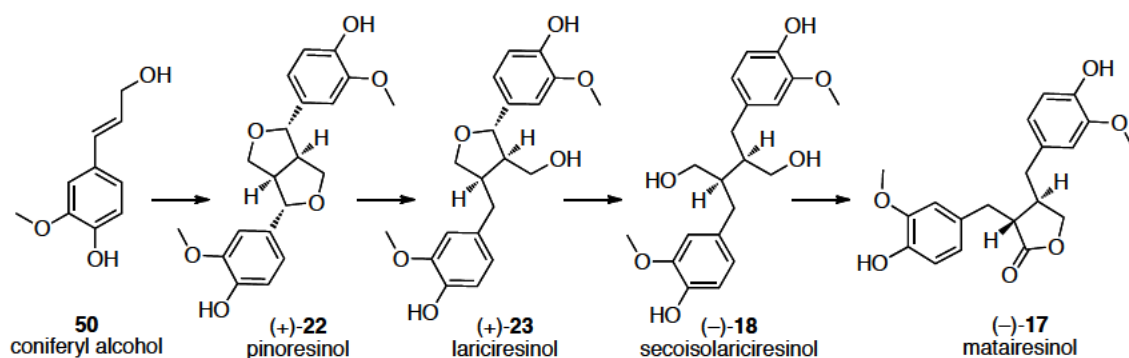


**Figure 9** A structural scheme for softwood lignin fragments.<sup>86</sup> The most frequent lignin inter-unit linkage is the  $\beta$ -O-4 linkage,<sup>87,88</sup> but there are also  $\beta$ - $\beta$  linkages (8,8') familiar from the lignan structures.



**Scheme 12** Biosynthesis of lignans and lignins stems from the shikimate-chorismate pathway and formation of the aromatic amino acids tyrosine **48** and phenylalanine **47**.<sup>14,84,85</sup>

Unlike lignin, the lignans are initially typically formed as optically active dimers, and they exist in diverse enantiomeric compositions, varying the particular antipode present with the plant species. Various enantiomeric mixtures (% e.e. values) and sometimes even racemates occur. Dibenzylbutyrolactone lignans, which occur late in the biosynthetic pathway, are in chiral liquid chromatography analyses found to be optically pure (>99 % e.e.) and levorotatory in most cases (Scheme 13).<sup>11</sup> On the other hand, the most biosynthetically upstream furofuran and furan lignans seem to be mixtures of both enantiomers and exhibit various enantiomeric compositions.<sup>10,11</sup>



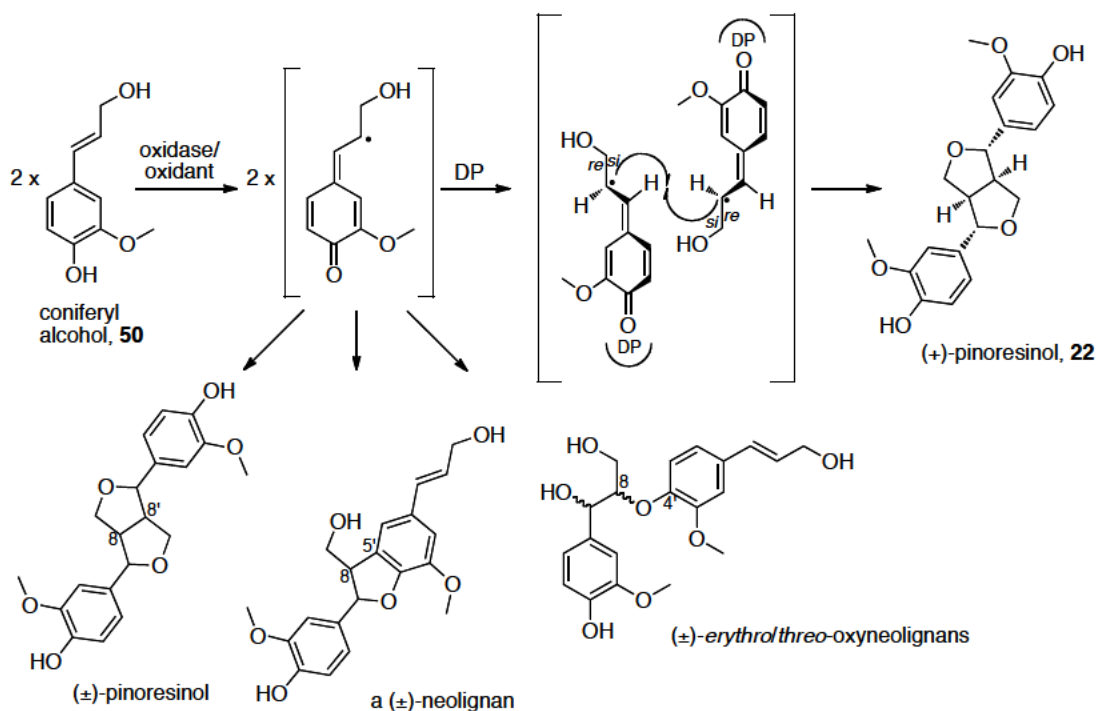
**Scheme 13** The conversion from coniferyl alcohol (50) to matairesinol 17 via pinoresinol 22, lariciresinol 23 and secoisolariciresinol 18 appears to be a common lignan biosynthetic pathway.<sup>11</sup> These lignans are sometimes called upstream lignans, and biosynthetic pathways of many other lignans can be regarded as starting from these four.<sup>10,89</sup>

The biochemical processes involving coupling and post-coupling modifications in lignans are under explicit control. The formation of dimers are highly stereoselective and/or enantiospecific controlled,<sup>84,85</sup> but there seems to be a great diversity in the stereochemical mechanisms of lignan biosynthesis in different plants. The coupling of phenylpropanoid units can be achieved by peroxidases and/or laccases, which form radicals that can dimerise by a radical coupling typically leading to racemic mixtures of the coupling products. As the lignans are formed as optically active dimers, it was discovered that when a so-called dirigent protein is present, the coupling occurs stereoselectively (Scheme 14). At first a (+)-pinoresinol-forming dirigent protein was found in *Forsythia* species,<sup>90</sup> and later more dirigent protein families and homologs have been found leading to the opposite antibodies.<sup>14,91</sup>

Although the dirigent protein is an asymmetric inducer, the enantiomeric compositions of pinoresinol 22 from various plant species vary largely. On the other hand, matairesinol 17 and the other dibenzylbutyrolactone lignans, whose formation is catalysed by various enzymes (e.g. pinoresinol/lariciresinol reductase and secoisolariciresinol dehydrogenase), are optically pure.<sup>10,11</sup> Thus it is suggested that the enantiomeric control of dirigent protein is not strong enough to give rise to optically pure pinoresinol, and a variation in the enantiomeric composition of the upstream lignans (lariciresinol, secoisolariciresinol and matairesinol) among different plant species may be ascribed, at least partly, to the selectivities of the pinoresinol/lariciresinol reductase and secoisolariciresinol dehydrogenase isoforms.<sup>92</sup>

The dirigent proteins that have been characterised to date are all involved in lignan synthesis.<sup>93</sup> A comparative mechanism “dirigent (or nucleation) sites” is postulated to be active in lignin formation, and there has been an intense debate whether macromolecular lignin configuration results from random or non-random radical coupling.<sup>14,88,94,95</sup> There are many recent findings in the general phenylpropanoid pathway and stereospecific biosynthetic enzymes are

identified, but not all biosynthetic steps, nor the regulation of the lignan biosynthetic pathways are fully understood.<sup>9,85,96</sup>



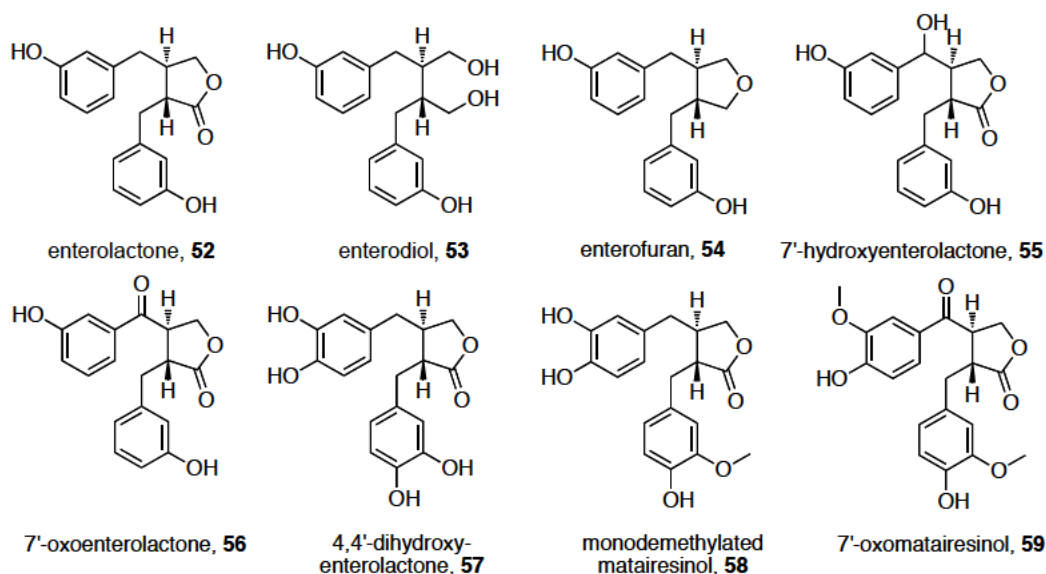
**Scheme 14** Stereoselective coupling of coniferyl alcohol radical intermediates at *si-si* faces in presence of (+)-pinoresinol-forming dirigent proteins (DP). Random coupling (without binding to dirigent proteins) leads to various racemic products.<sup>14,91</sup>

## 3.2 Mammalian lignans

At the beginning of 1980s, two new lignan compounds were identified from human and animal species.<sup>1,2</sup> Their structures were shown to be *trans*-2,3-bis(3-hydroxybenzyl)- $\gamma$ -butyrolactone and 2,3-bis(3-hydroxybenzyl)butane-1,4-diol<sup>97</sup> (or *trans*-lignano-9,9'-lactone and -diol according to IUPAC). Later they were given trivial names enterolactone **52** and enterodiol **53**, respectively.

Since then new mammalian lignans have been found and identified as plant lignan metabolites, e.g. enterofuran **54** and 7'-hydroxyenterolactone **55**,<sup>98</sup> 7'-oxoenterolactone **56**, 4,4'-dihydroxyenterolactone **57**, monodemethylated matairesinol **58** and 7'-oxomatairesinol **59** (Figure 10).<sup>82</sup> In recent years no new metabolites have been reported.





**Figure 10** Mammalian lignans.

From a structural point of view, mammalian lignans differ from plant lignans in their aromatic substitution pattern. Mammalian lignans are not necessarily *para*-substituted, but have a phenolic hydroxy group in the *meta*-positions. Such aromatic substitution pattern is not generally found in plant lignans.

The mammalian lignans are formed from the plant lignan (glycoside) precursors by the activity of the gastrointestinal microbiota. Intestinal bacteria metabolise plant lignans by different processes, such as deglycosylation, ring cleavage, demethylation, dehydroxylation, oxidation, etc. Epidemiological and pharmacological studies have revealed that, due to their structural similarities with estrogens, mammalian lignans, enterolactone **52** in particular, afford protection against osteoporosis, breast, colon and prostate cancers, cardiovascular diseases, and menopausal syndrome.<sup>99</sup> The mechanism of protection against various diseases are probably different, but mostly unknown. It has been also suggested that enterolactone could merely be a biomarker of a healthy lifestyle,<sup>100</sup> but in recent studies some of previously unknown mechanisms have been revealed and enterolactone has shown an active role in e.g. inhibiting breast cancer growth.<sup>101,102</sup>

Recently, mammalian lignans enterolactone **52** and enterodiol **53** have been detected at low concentrations in herbs,<sup>103</sup> but as they were found also in different water samples, it is more likely that they have entered the plant exogenously (uptaken from the water surrounding the roots) instead of produced endogenously,<sup>104</sup> since no such biosynthetic pathway has yet been reported.

### 3.3 Dietary lignans

Due to their ubiquitous presence, lignans are included in our daily diet. Being fiber-related polyphenols,<sup>105</sup> they are present at considerable concentrations in fiber-rich foods, such as whole grain products, cereals and seeds, but also in fruits, berries, vegetables, and legumes. However, the highest lignan concentrations have been found in oilseeds such as flaxseed,<sup>106</sup> linseed and sesame seed.<sup>107</sup> At the ecological level lignans, having antimicrobial, antifungal, insecticidal and antioxidant properties among others, may have a role in the plant defence. Lignans have also various biological effects, e.g. anticancer and antiviral effects, and influence on gene expression (activation).<sup>108,109,110</sup> Originally only secoisolariciresinol **18** and matairesinol **17** were identified as precursors of the mammalian lignans enterolactone **52** and enterodiol **53**.<sup>111</sup> Later several other precursors were found, such as pinoresinol **22**, syringaresinol **60**, lariciresinol **23**, arctigenin **61** and 7'-hydroxmatairesinol **31**.<sup>81,98,107</sup>

Already in the early 1980's it was suggested that a diet rich in lignans and other phytoestrogens may protect against a range of chronic western diseases, such as breast cancer and osteoporosis. The connection between diet and diseases was originally introduced, because in Asian countries, where soybean products are staple food, there was until recently a very low incidence of hormonal cancers and coronary heart disease. Nowadays, particularly in Japan the incidence is increasing due to a change in dietary habits, and the disease risk has also observed to increase after immigration from Asia to United States and going native in diet. Numerous studies have been conducted to evaluate the hypothesis, that consumption of fiber-rich, low-fat diet is linked with a lower risk of breast cancer and other estrogen-related diseases.<sup>112,113</sup> It has been suggested that this may be related with the high intake of fiber-associated plant lignans that are converted to mammalian lignan enterolactone **52**. In several case-control epidemiological studies, an inverse correlation between serum and urine enterolactone concentrations and breast cancer risk has been found, while in some studies no correlation was found. In most of the recent cohort studies, the correlation between enterolactone and breast cancer risk has remained unclear.<sup>100,112,114,115</sup> However, in recent meta-analyses, combining results of several cohort and case-control studies, plant lignans have shown a possible association with reduced breast cancer risk in postmenopausal women.<sup>116,117</sup>

To estimate lignan intake in various populations and demonstrate the association between the lignan containing food and risk of developing chronic diseases, lignan contents of foods in different countries have been quantified.<sup>106,118,119,120</sup> Previously whole grains were considered the most important lignan sources. When more dietary lignans are included in these databases, it has become clear that some vegetables and fruits are as important lignan sources as whole grains. However, in countries like Finland, rye bread contributes



significantly to the intake of lignans because of its relatively high consumption.<sup>120</sup>

The reported lignan compositions and concentrations in different studies are greatly dependent on the sample pre-treatment applied and on the analytical method used, and variations are observable even when using same sample preparation and analytical methods.<sup>121</sup> Thus it is possible that the content of lignans and the conjugation patterns of individual lignans may vary within the same species depending on natural variation, such as genetic factors or growth conditions.<sup>81,107</sup> It also seems that as the research is increasing, new different precursors are continuously found. In many studies only a few lignans have been determined, probably due to a lack of standards. Previously only matairesinol **17** and secoisolariciresinol **18** were quantified, and they were considered the major lignans in rye,<sup>122</sup> but later pinoresinol **22**,<sup>98</sup> and syringaresinol **60**<sup>123</sup> were found to be the more abundant precursors. Some lignans are acid labile, and others are of limited stability under alkaline conditions. Different conjugated forms require different hydrolysis conditions. Hence, the establishment of one universally applicable method for analysis of a broad spectrum of lignans in various matrices is likely too demanding, and it is necessary to employ complementary sample preparation methods.<sup>121</sup>

Natural variations, identity of lignan glycosides, enantiomeric compositions and oligomeric or polymeric derivatives of lignans have been studied to a very limited degree.<sup>107</sup> It is known that even a small difference in a plant lignan structure results in marked changes in biological activity *in vivo* as well as in lignan metabolism.<sup>124,125</sup> Thus the study is just beginning.

### 3.4 Metabolism of dietary lignans

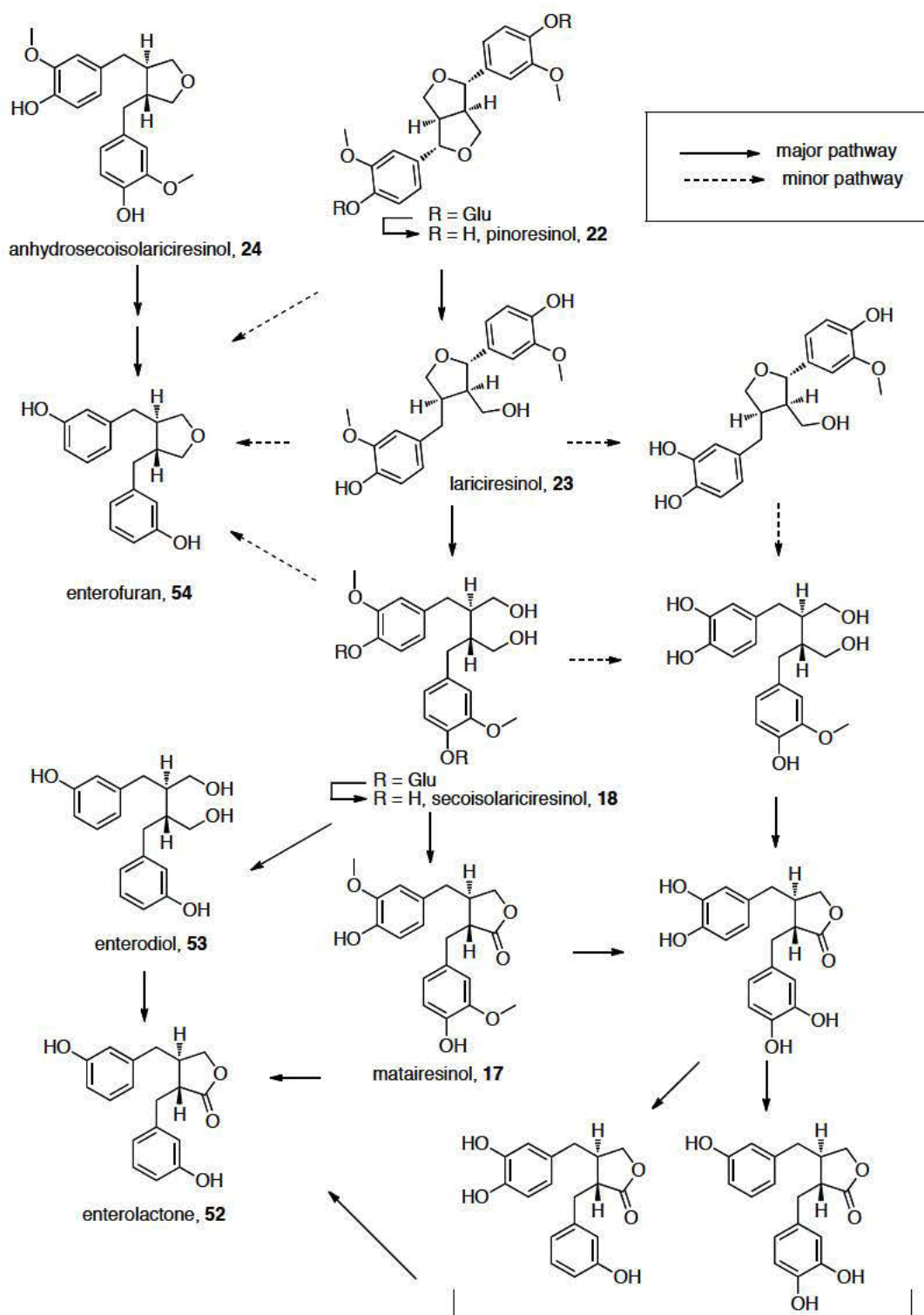
The metabolism of dietary lignans is under continuous investigation. The mammalian lignans are formed from plant lignans and their glycosides in the human body in the gastrointestinal tract. The bacteria hydrolyse the possible sugar moiety of the plant lignan glycosides to release the plant lignan. The more hydrophilic lignan precursors (such as secoisolariciresinol diglucoside) are readily metabolised to enterodiol **53** and enterolactone **52** by the intestinal microbiota before absorption, while some other lignans (such as sesamin) are absorbed and metabolised in the liver, excreted in bile and undergo enterohepatic circulation, and then further metabolised to mammalian lignans by the intestinal microflora.<sup>99</sup> The path from secoisolariciresinol **18** and matairesinol **17** to enterolactone is well elucidated, but overall there are many unsolved aspects in the complex phenomena in mammals.

The biosynthesis of matairesinol **17** and secoisolariciresinol **18** in plants has been reported to occur by the breakdown of pinoresinol **22** to secoisolariciresinol via lariciresinol **23**. The intestinal bacterial metabolism of pinoresinol seems to occur in a similar way. The hydrolysis of lignan glycosides to their aglyconic forms is the first step (Scheme 15). Second, the demethylation

of a methoxy group adjacent to an aromatic hydroxy group takes place easily, while two vicinal methoxy groups are rather resistant to demethylation. Third, 4-OH-dehydroxylation which takes place leaving the 3-hydroxy group on the phenyl ring. Such pattern, present in e.g. mammalian lignans, appears to be stable and resistant to further dehydroxylations. The oxidation of dibenzylbutanediols to dibenzylbutanelactone may occur at each step.<sup>98,126,127</sup>

Most of the lignans are chiral due to their chemical structure, and possess at least one pair of enantiomers. The dietary lignans occur in different stereochemical forms.<sup>107</sup> So do the mammalian lignans enterolactone **52** and enterodiol **53**, having two enantiomeric, mirroring forms (8*R*,8'*R*)-(-) and (8*S*,8'*S*)-(+). The absolute configurations at the chiral carbons 8 and 8' seem not to change during the various reactions performed by intestinal bacteria.<sup>127,128</sup> However, the first found mammalian lignans were racemic,<sup>1,129</sup> which might indicate that the net formation of both enantiomeric forms of mammalian lignans is close to a racemic mixture.

Different studies have analysed the human intake of lignans, but so far it is unknown what proportion of the ingested plant lignans is metabolised in the gut, and absorbed finally reaching the target issue.<sup>99</sup> The serum enterolactone concentration varies widely in the population, and the consumption of lignan containing foods explains only a small part of the variation.<sup>130</sup> Inter-individual differences in cell densities of mammalian lignan producing bacteria may explain the differences in blood concentrations of enterolignans.<sup>99</sup> Use of antibiotics lowers the serum enterolactone concentrations,<sup>131</sup> which support the suggestion of a crucial role for the gut in the metabolism of lignans.<sup>132</sup> However, there are several variables complicating the study and to be taken into account: gender, age, body mass index, diet, nutrition (when used questionnaires, people may overestimate their consumption of vegetables and other healthy foods), use of antibiotics, smoking, etc. may affect.<sup>133</sup> In addition to the most studied enterolactone and enterodiol, there might be other relevant metabolites, and also the plant lignans may have health benefiting properties as such.<sup>96,134,135,136</sup>



**Scheme 15** Proposed metabolism of representative plant lignans to mammalian lignans.<sup>98,126</sup>

## 4 Stable isotope labelling and lignans

Lignans may be labelled with isotopes of carbon, oxygen and hydrogen depending on their elemental composition. In this section the labelling of lignans with deuterium, the stable, heavy isotope of hydrogen is reviewed. Also the syntheses of stable carbon-13 and oxygen-18 labelled lignan analogues are briefly discussed. Radioactive tritium and carbon-14 labellings are excluded from this thesis.

### 4.1 Stable isotope labelled compounds

The interest in stable isotope labelled compounds has resulted from the numerous application of mass spectrometry as a specific detection tool in biomedical, pharmacological, and environmental analysis. In addition to metabolic<sup>89,124,137</sup> and analytical studies,<sup>138,139,140</sup> isotopically labelled analogues are required also for reaction mechanistic studies<sup>141</sup> and structural elucidation.<sup>142</sup>

The advances in the knowledge of the absorption and the metabolism of lignans and other dietary phenols have been associated especially with the development of sophisticated liquid chromatography–mass spectrometry (LC–MS) techniques. However, the phytoestrogen contents are usually very low in the biological matrices, and these techniques remain largely qualitative unless authentic standards are available. Thus, isotopically and isomerically pure polydeuterated lignans and other phytoestrogens are needed as references for quantitation of these compounds in biological samples. The use of structural analogues as internal standards, rather than authentic isotope labelled isotopologues,<sup>†</sup> is undesirable because they will have different behavior in sample preparation as well as different retention times and ionization properties compared with the analytes. An authentic stable isotope labelled analogue of a compound is identical to the compound of interest except for mass.

The most common techniques for the determination of lignan contents are liquid chromatography (LC) and gas chromatography (GC) with various detection systems. By far the predominant and most popular applications for analysing lignans and other phytoestrogens are the (HP)LC–MS methods. In contrast to GC–MS, LC–MS has the advantage that it is not necessary to prepare volatile derivatives and that conjugated forms, such as the glycosides, can be analysed as easily.<sup>121,143,144</sup>

One of the most reliable and sensitive method of quantification is based on the addition of a stable isotope analogue of the analyte of interest into a sample

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<sup>†</sup> Isotopologues are substances that differ only in isotopic composition, e.g. CH<sub>3</sub>OH, CD<sub>3</sub>OH, CH<sub>2</sub>DOH.

at a known concentration. The technique is called stable isotope dilution (SID).<sup>145</sup> Since the analyte and its isotopologue possess almost identical chemical and physical properties,<sup>‡,146</sup> the isotopic ratio remains stable as they are affected equally by variations at extraction, sample preparation, injection and instrument parameters.<sup>147</sup> In the ion chromatogram, the analyte and its labelled analogue usually have the same retention time. Thus, all the losses during sample preparation can be corrected for and the analytes reliably identified and quantified.

#### 4.1.1 Requirements for the internal standards

In quantitation methods, internal standards are used to take into account all the losses during the process and the behaviour of the compounds during the measurement. A stable, chemically, isomerically and isotopically pure isotopologue of the analyte is an optimal standard. As the physicochemical properties of an isotope labelled analogue are practically identical with those of the analyte, no separation takes place during the process: the analyte and its labelled analogue have the same retention times in chromatography but different  $m/z$  value in mass spectrometry.

Labelled compounds that are used as internal standards in quantitative analytical techniques such as GC-MS and LC-MS must fulfil certain important criteria. First, the compounds and the labels must remain stable during the entire sample preparation procedure and analysis. Second, there can not be any unlabelled species present in the standard. And third, the labelled analogue and the analyte should have a difference of at least three units in the  $M^+$   $m/z$  value in order to avoid the internal standard peaks overlapping those of the analytes.<sup>148</sup> On the other hand, it is claimed that for internal standards an optimal number of deuteriums would be from three to five, and more deuteriums in the molecule may cause chromatographic separation between internal standard and the analyte.<sup>149,150</sup> However, no chromatographic separation of a deuterated compound from the corresponding unlabelled compound has been reported for lignans.

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<sup>‡</sup> Isotopes do differ. Due to the different masses, deuterium and hydrogen (other isotopes as well) have different zero-point energies. The zero-point energy differences produce so called isotope effects: steric effect (the C-D bond length is shorter than the C-H bond length); inductive effect (the C-D bond is more polar than the C-H bond); hyperconjugative effect (the C-D bond is less polarisable than C-H bond),<sup>151,160</sup> and kinetic isotope effect. C-D bond is marginally stronger than C-H bond, and reactions in which C-H bonds break may go (in theory at room temperature 7 times) faster than reactions in which C-D bonds break, if the bond to H (or D) is involved in the rate-determining step.

Thus isotopes have different physical properties, e.g. on their nuclear spin, which affects how they behave in NMR. Since IR stretching frequencies depend on mass, C-D bonds have lower stretching frequencies than C-H bonds. And as some chemical properties depend on atomic mass, isotopes may also differ chemically. The difference is considered more or less significant only for hydrogen, since no other element has one isotope twice as big as another.<sup>146</sup>

Isotopic purity is an important feature of an isotope labelled compound. However, in the literature and in the catalogs of commercial suppliers there seems not to be any established practice on how to define or measure the isotopic purity, isotope distribution or isotope content. In this thesis and in the current work, the isotope distribution is determined by NMR and the isotopic purity is indicated as the percentage of the most abundant *d*-species, determined from the molecular ion region in EI mass spectra by comparison with those of the undeuterated compounds (as will be discussed in more detail in Chapter 6.2.4).

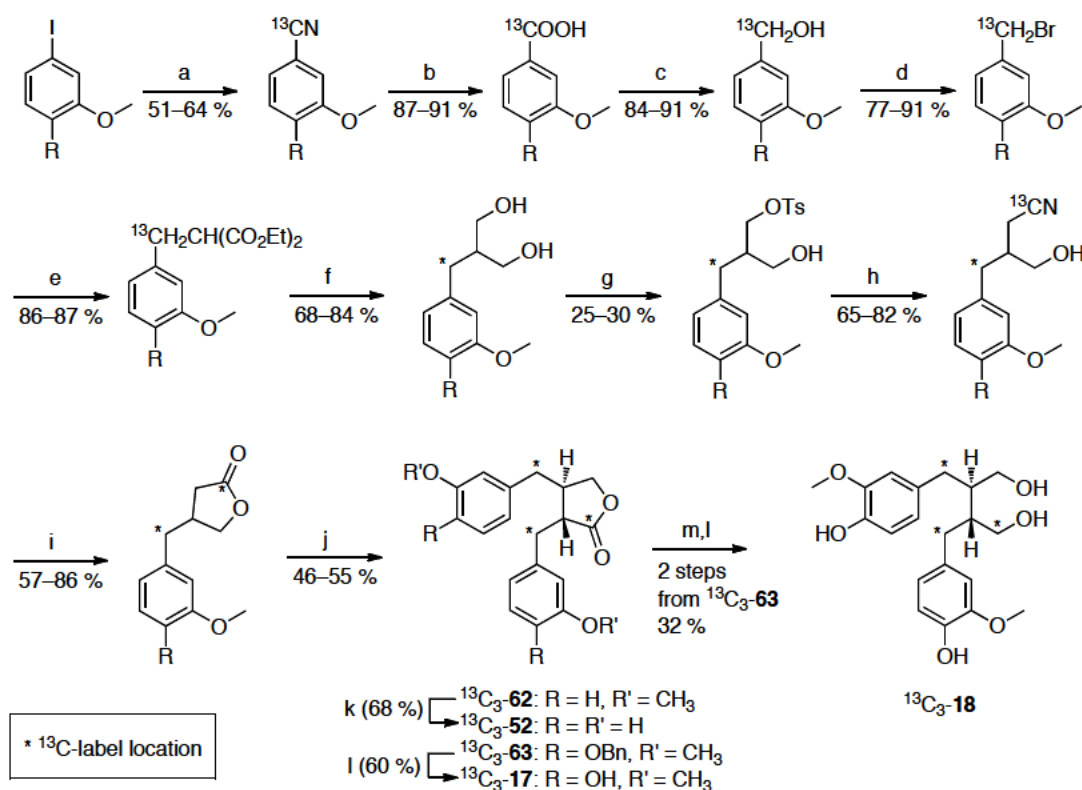
#### 4.1.2 Labels for lignans

Possible labels for lignans are  $^{18}\text{O}$ ,  $^{13}\text{C}$  and deuterium, the stable isotopes of oxygen, carbon and hydrogen, respectively.  $^{18}\text{O}$ -labelled compounds are used to study reaction mechanisms,<sup>1,151</sup> but they could also be used as stable isotope labelled analogues for quantitative determinations.  $^{16}\text{O}/^{18}\text{O}$  exchange is limited to compounds such as carboxylic acids that contain functional groups prone to facile oxygen exchange reactions.<sup>152</sup> Oxygen labelling has been used also when reactive phenols, e.g. resorcinol moieties, are present, but the labelling conditions required were quite harsh (180 °C for 16 h), the yields were low, and protective groups were needed.<sup>153</sup> For the best of our knowledge, no oxygen-18-labelled lignans have been synthesised apart from our mechanistical studies.<sup>1</sup>

In the literature there are some examples of carbon labelled lignan precursors,<sup>154</sup> that have been used to examine the biosynthesis and formation mechanisms of lignans and neolignans.<sup>137,141,155</sup> The non-radioactive isotope of carbon,  $^{13}\text{C}$ , might be considered as an optimal, general purpose label since the isotope effects with  $^{13}\text{C}$  are negligible (compared to  $^{18}\text{O}/^{16}\text{O}$  and D/H), it can be used to label the considerably stable carbon backbone of lignans and it cannot be exchanged for  $^{12}\text{C}$  under ordinary conditions. However, the stability is paralleled by the required total or partial synthesis using expensive  $^{13}\text{C}$  labelled reagents or starting materials. There is only one total synthesis of stable  $^{13}\text{C}$  labelled lignan derivatives.<sup>156,157</sup> The synthesis of carbon-13 labelled enterolactone  $^{13}\text{C}_3$ -**52**, matairesinol  $^{13}\text{C}_3$ -**17** and secoisolariciresinol  $^{13}\text{C}_3$ -**18** is shown in Scheme 16. Since the first  $^{13}\text{C}$  label is introduced, it takes 11 or 12 steps to get the final triple labelled target, and although the yields for most of the steps are very good, the overall yields fall to 0.3–2 %.<sup>156,157</sup>

On the other hand, deuterium labelling of organic compounds provides the by far most important route to stable isotope labelled standards. This result from the ubiquitous distribution of hydrogen in the target material, the ease of H/D exchange in many cases, and the cheap and readily available deuterated solvents and reagents. Furthermore, depending on the functional groups of the target molecules, it is often possible to acquire the desired label without any synthesis but performing simple exchange reactions on the native molecules in deuterated solvents.<sup>152</sup> The various deuteration reagents and solvents such as

D<sub>2</sub>O, CH<sub>3</sub>OD, DCl, D<sub>2</sub>SO<sub>4</sub> and NaOD for deuterium exchange and e.g. the reducing agents LiAlD<sub>4</sub> and NaBD<sub>4</sub>, used in total synthesis approaches, are readily available and less costly than <sup>18</sup>O and <sup>13</sup>C labelled reagents.



**Scheme 16** Reagents and conditions: a) K<sup>13</sup>CN, Ca(OH)<sub>2</sub>, Pd(OAc)<sub>2</sub>, DMF; b) 2 M NaOH, reflux; c) LiAlH<sub>4</sub>, Et<sub>2</sub>O; d) TMSBr or PBr<sub>3</sub>, Et<sub>2</sub>O; e) KO<sup>t</sup>Bu, CH<sub>2</sub>(CO<sub>2</sub>Et)<sub>2</sub>, glyme; f) LiAlH<sub>4</sub>, Et<sub>2</sub>O; g) *p*-TsCl, Et<sub>3</sub>N, CH<sub>2</sub>Cl<sub>2</sub>; h) K<sup>13</sup>CN, 18-crown-6, MeCN; i) 2 M NaOH, reflux; j) LDA, <sup>13</sup>C-labelled benzyl bromide or iodide, THF, -78°C; k) BBr<sub>3</sub>, CH<sub>2</sub>Cl<sub>2</sub>; l) H<sub>2</sub>, Pd/C; m) LiAlH<sub>4</sub>, THF. R = H; OBn.<sup>157</sup>

Resulting from the isotope effect, deuterated analogs are not the most ideal internal standards. There may be a small separation of the deuterated internal standard and its endogenous protium form during chromatography.<sup>158</sup> Ion fragmentation is sensitive to the kinetic isotope effect, but differences in ionization efficiency between labelled and unlabelled compounds are very small and become unmeasurable when the labelling site is remote to the charge center, even in the case of deuterium.<sup>159</sup> Yet, structural analogs are even less representative of the endogenous compounds since, in addition to differences in retention time, the structural analog can show different absorption loss.<sup>158</sup> Thus deuteration has remained the most important exchange reaction and labelling method to obtain stable isotope labelled compounds.

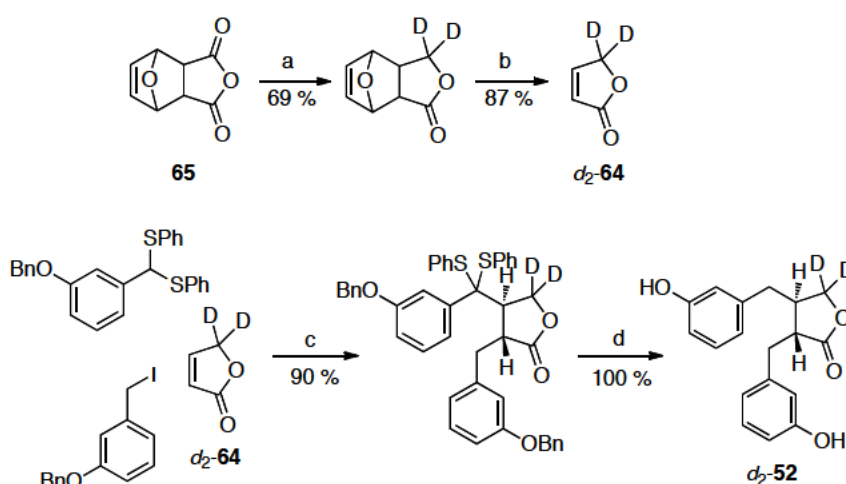
A comprehensive book concerning deuterium labelling in organic chemistry was published more than 40 years ago,<sup>160</sup> and a couple of more recent general reviews on the preparation of stable isotope labelled compounds, their use as internal standards<sup>147,152</sup> and hydrogen isotope exchange reactions are

available.<sup>161</sup> A recent review compiles advances in the synthetic labelling and biolabelling of lignans and other polyphenols since year 2000. It covers both stable and radiolabels of hydrogen and carbon isomers.<sup>162</sup>

## 4.2 Synthesis of deuterated lignans

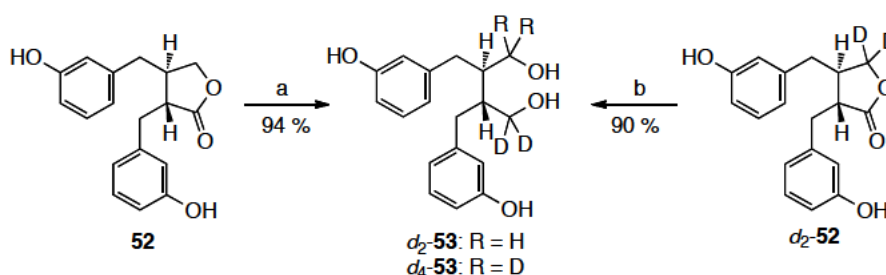
### 4.2.1 Introduction of deuteriums via reduction

The first reported synthesis for deuterium labelled lignans was a multistep total synthesis for  $d_2$ -enterolactone,  $d_2$ - and  $d_4$ -enterodiols.<sup>163,164</sup> The method was based on Pelter's *et al.* synthesis of *trans*-dibenzylbutyrolactones via tandem conjugate addition.<sup>165</sup> The Michael addition-alkylation method was modified by using  $d_2$ -butenolide  $d_2$ -**64** as starting material to produce  $d_2$ -**52** (Scheme 17).



**Scheme 17** The first reported deuterolabelled lignan.<sup>163</sup> Reagents and conditions: a)  $\text{NaBD}_4$ , DMF, 0 °C; b) 16 mmHg, 200 °C; c) i)  $n\text{-BuLi}$ , THF, -78 °C, ii) TMEDA; d) Raney-Ni, EtOH, reflux.<sup>164</sup>

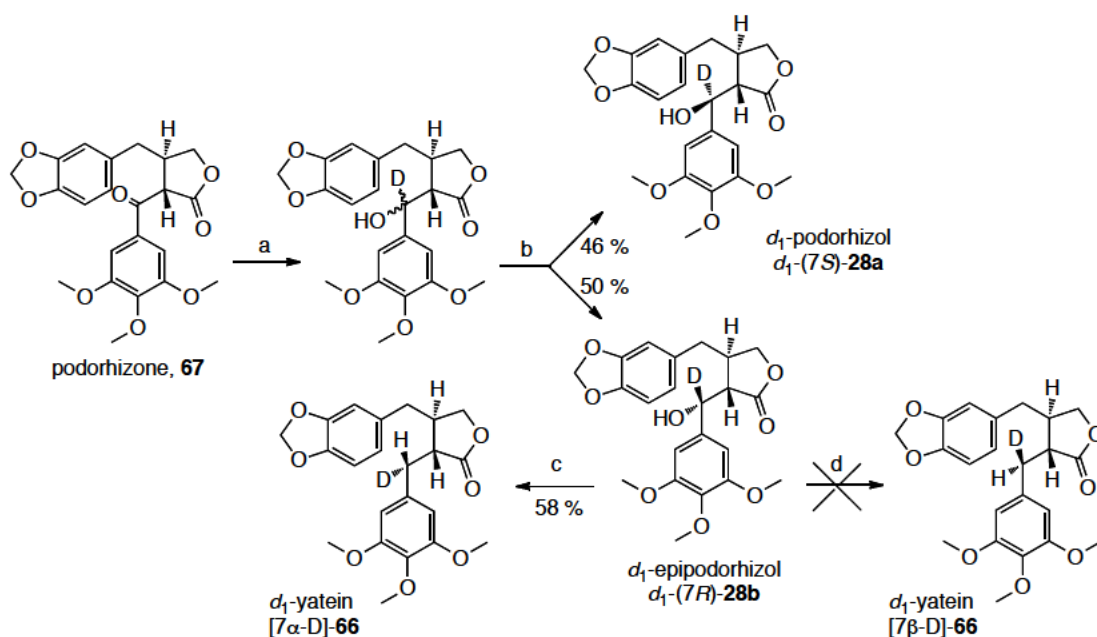
Deuterium labelled enterodiols were available in two forms:  $d_2$ -**53**, either by reducing unlabelled enterolactone **52** with  $\text{LiAlD}_4$  or by reducing labelled enterolactone  $d_2$ -**52** with  $\text{LiAlH}_4$ , and  $d_4$ -**53** by reducing the labelled lactone  $d_2$ -**52** with  $\text{LiAlD}_4$  (Scheme 18).



**Scheme 18** Reagents and conditions: a)  $\text{LiAlD}_4$ , THF, R = H; b)  $\text{LiAlD}_4$ , THF, R = D.<sup>164</sup>



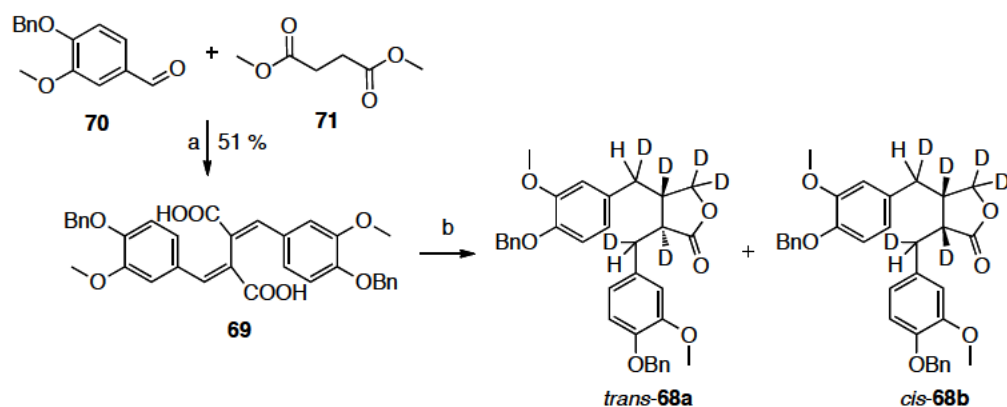
Neidigh *et al.* synthesised monodeuterolabelled podorhizol  $d_1$ -**28a**, epipodorhizol  $d_1$ -**28b**, and the other of the yatein isotopomers<sup>§</sup>  $d_1$ -**66**,<sup>166</sup> in order to later study the biosynthesis of podophyllotoxin (Scheme 19).



**Scheme 19** Synthesis of monodeuterated podorhizol  $d_1$ -**28a**, epipodorhizol  $d_1$ -**28b** and yatein  $d_1$ -**66**. The proposed preparation (d) of the other yatein isotopomer failed to yield isolable amounts of the desired compound. Reagents and conditions: a) NaBD<sub>4</sub>, EtOH, 0 °C, 2 h; b) chromatographic separation (total yield 96 %); c) Pd/C (5 %), H<sub>2</sub>, EtOAc, H<sup>+</sup>, 48 h; d) Raney-Ni, H<sub>2</sub>, AcOEt, H<sup>+</sup>.<sup>166</sup>

A two-step synthesis<sup>167</sup> of symmetrically substituted butyrolactone lignans was applied to prepare hexadeuterated benzyl protected derivatives of matairesinol **68** (Scheme 20).<sup>168</sup> The first step is the formation of a fulgenic acid **69** by the Stobbe condensation of 4-benzyloxy-3-methoxybenzaldehyde **70** and dimethyl succinate **71**. The second, deuteration step is a ruthenium-catalysed ring-closing with simultaneous deuteration, giving benzyl protected  $d_6$ -matairesinol derivatives *trans*-**68a** and *cis*-**68b** in a 10:1 ratio (yield of the reaction was not mentioned).

<sup>§</sup> Isotopomers (or isotopic isomers) are molecules that have the same numbers of each type of isotopic atom but in different arrangements in the molecule, e.g. CH<sub>2</sub>DOH and CH<sub>3</sub>OD.



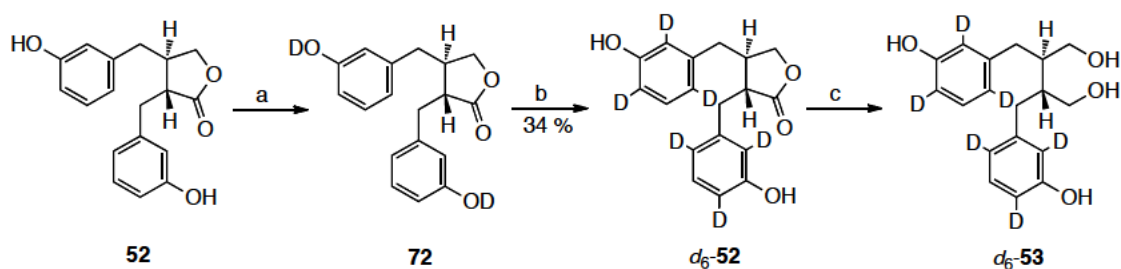
**Scheme 20** Reagents and conditions: a) NaH, toluene; b) D<sub>2</sub>, Ru<sub>4</sub>H<sub>4</sub>(CO)<sub>8</sub>(PBu<sub>3</sub>)<sub>4</sub>, 48 h, 180 °C.<sup>168</sup>

#### 4.2.2 Labelling via H/D exchange of aromatic protons

All the other syntheses for deuterium labelled lignans are based on H/D exchange within the complete molecular framework. In the case of lignans, the exchangeable protons are in the aromatic rings.

The first efforts to deuterate aromatic compounds were in 1934, three years after discovering the heavy hydrogen isotope.<sup>169</sup> Horiuti and Polanyi heated benzene in 3 % heavy water in the presence of nickel catalyst.<sup>170</sup> Later in the same year Ingold *et al.* introduced deuterium into the aromatic nucleus by means of ordinary electrophilic reagents, namely concentrated aqueous sulphuric acid (without heterogenous catalysis).<sup>171</sup> They also proposed that the mechanism of the exchange is an electrophilic aromatic substitution,<sup>172</sup> and proved the theory with comparison of the efficiencies of some acidic and basic deuterating agents and of the influence of some aromatic substituents.<sup>173,174</sup> In lignans the aromatic substituents usually are hydroxy and/or methoxy groups.<sup>8</sup> When an electrophilic substitution reaction, such as deuteration, is performed, the existing substituents determine which position the new group will take and whether the reaction will be slower or faster than with benzene. Both hydroxy and methoxy are *ortho*–*para* directing and activating groups.<sup>175</sup>

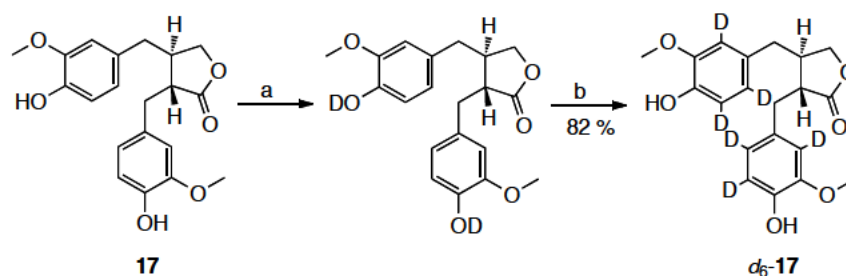
The first deuterium exchange within a lignan skeleton was performed with DBr (PBr<sub>3</sub> in D<sub>2</sub>O, Scheme 21).<sup>176</sup> The protons of phenolic groups were replaced with deuterons by D<sub>2</sub>O treatment before deuteration procedure, and the predeuterated enterolactone **72** was labelled with deuterium by exchange in DBr–D<sub>2</sub>O to give the hexadeuterated enterolactone *d*<sub>6</sub>-**52**. Aromatic protons that are *ortho* or *para* to the directing and activating phenolic OH groups were exchanged, while the protons which are *meta* to OH remained unaffected. The H/D treatment was repeated to ascertain complete deuteration and over 90 % isotopic purity. After final exchange the reaction products were treated with large excess of H<sub>2</sub>O or ethanol to restore the protic hydroxy groups.



**Scheme 21** Reagents and conditions: a)  $\text{D}_2\text{O}$ ; b)  $\text{PBr}_3$ ,  $\text{D}_2\text{O}$ , reflux, 5h (35 %); c)  $\text{LiAlH}_4$ , THF.<sup>176</sup>

Deuterated phosphoric acid  $\text{D}_3\text{PO}_4$  was used to synthesise  $d_6$ -derivative of matairesinol (Scheme 22).<sup>138</sup> Matairesinol **17** was first predeuterated with  $\text{CH}_3\text{COOD}$ , made from freshly distilled acetic anhydride by slow addition to heavy water and stirring the solution under argon for 2 h. The phenolic protons were changed to deuteriums by dissolving matairesinol in labelled acetic acid, leaving it stand for several hours and finally evaporating the solvent. The labelled phosphoric acid  $\text{D}_3\text{PO}_4$  was prepared by adding  $\text{D}_2\text{O}$  to  $\text{P}_2\text{O}_5$ . Predeuterated matairesinol was added to deuterated phosphoric acid under argon, and the reaction mixture was stirred at  $80^\circ\text{C}$  for 3 days. Deuteration and work-up was repeated three times. The isotopic purity was estimated from the mass spectrum to be 95 % for  $d_6$ -**17** and 5 % for  $d_5$ -derivative.

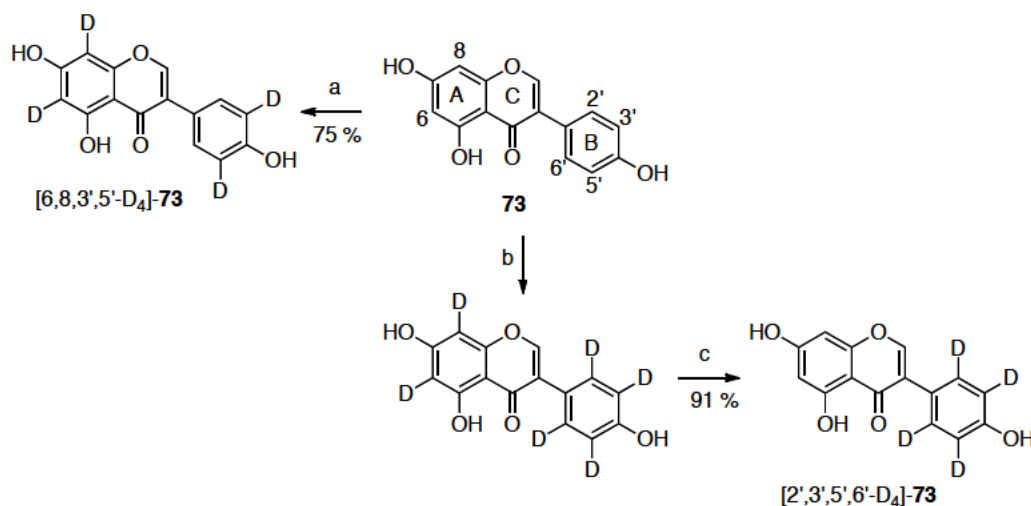
Neidigh *et al.* prepared a less than 20 milligram portion of hexadeuterolabelled matairesinol  $d_6$ -**17** by stirring the unlabelled lignan with  $\text{DClO}_4$  (68 % in  $\text{D}_2\text{O}$ ) in THF/ $\text{CDCl}_3$  at room temperature in only four hours.<sup>166</sup>



**Scheme 22** Reagents and conditions: a)  $\text{CH}_3\text{COOD}$ ; b)  $\text{D}_3\text{PO}_4$ ,  $80^\circ\text{C}$ , in total 9 days.<sup>138</sup>

An effective method using the deuterated phosphoric acid–boron trifluoride complex  $\text{D}_3\text{PO}_4 \cdot \text{BF}_3 / \text{D}_2\text{O}$  was developed in our laboratory initially to synthesise a stable deuterium labelled isoflavone genistein,<sup>177</sup> and later also other related compounds.<sup>178,179,180</sup> Originally tetradeutrogenistein was synthesised by refluxing predeuterated genistein **73** in  $\text{CF}_3\text{COOD}$  for two days to get  $d_4$ -genistein [6,8,3',5'- $\text{D}_4$ ]-**73** in 75 % yield and over 90 % isotopic purity (a in Scheme 23).<sup>138</sup> However, it was found out later that the most highly activated 6- and 8-site labels were not stable in the ID–GC–MS–SIM analytical procedure, but were lost to some extent making the [6,8,3',5'- $\text{D}_4$ ]-**73** unsuitable as an internal

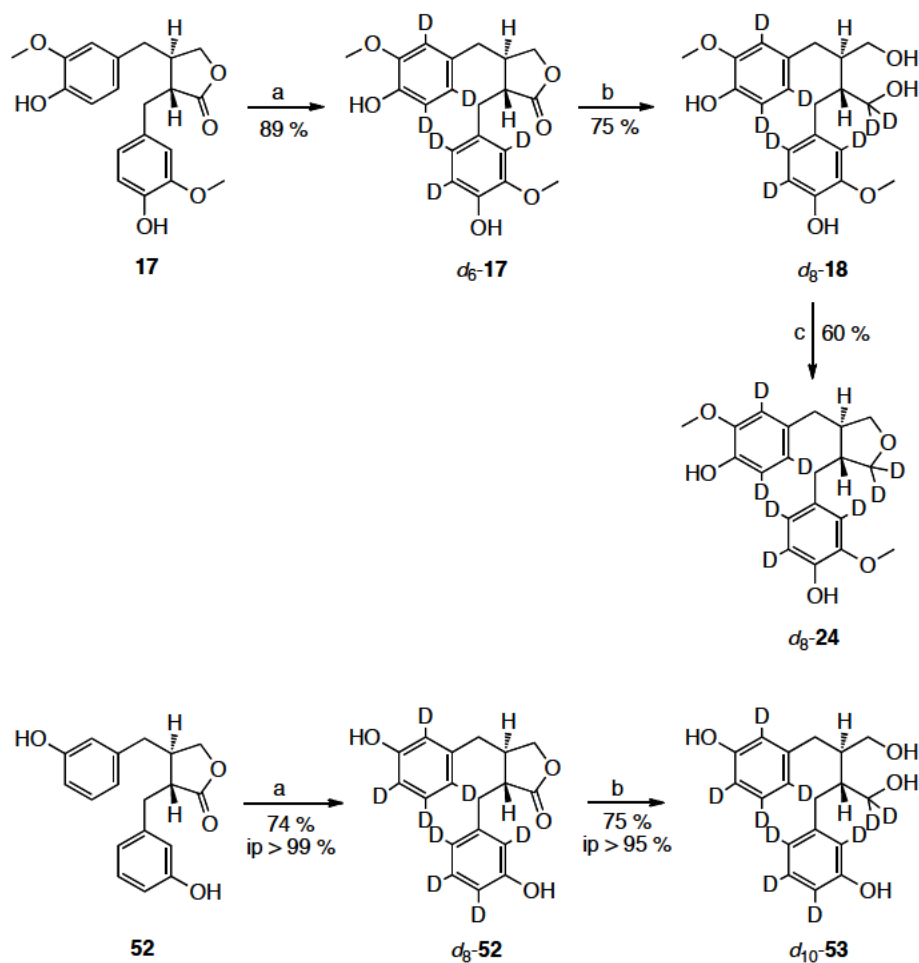
standard. In fact, the labile deuterium labels at sites 6 and 8 were readily exchanged back to hydrogens, and the two remaining deuteriums at sites 3' and 5', although stable, were not enough to avoid the interference of fairly intense  $M+1$ ,  $M+2$  and  $M+3$  ions of the natural isotopes of the unlabelled compounds in mass spectra (see requirements for the internal standards in Chapter 4.1.1). Thus several reagents were tested to overcome this problem. For example  $D_3PO_4$ , which was used for deuteration of matairesinol **17** to good effect, could exchange only a third of the ring A protons and none from the B ring. However, the deuterated phosphoric acid–boron trifluoride complex  $D_3PO_4 \cdot BF_3 / D_2O$  was active enough to exchange all the aromatic ring protons (b in Scheme 23).<sup>177</sup> After the removal of the labile 6- and 8-labels (c in Scheme 23) an isotopically pure and stable  $[2',3',5',6'-D_4]$ -isotopologue of tetradeuterogenistein  $d_4$ -**73** was obtained.<sup>181</sup>



**Scheme 23** Reagents and conditions: a)  $CF_3COOD$ ; <sup>138</sup> b) i)  $D_2O$ /acetone, ii)  $D_3PO_4 \cdot BF_3 / D_2O$ , 55 °C, 3 days, iii)  $H_2O/EtOH$ ; c) 1 %  $CH_3COCl$  in MeOH, reflux, 30 min. <sup>177</sup>

The deuteration reagent  $D_3PO_4 \cdot BF_3 / D_2O$  was then used also to deuterate the lignans matairesinol **17** and enterolactone **52** (Scheme 24). Treatment of matairesinol **17** worked as expected to give the hexadeuterated matairesinol  $d_6$ -**17** even much faster than with mere  $D_3PO_4$  (Scheme 22), i.e. in 20 hours instead of 9 days. The  $d_6$ -**17** was further reduced with  $LiAlD_4$  to give  $d_8$ -**18**, which was dehydrated under acidic conditions to the tetrahydrofuran lignan derivative anhydrosecoisolariciresinol  $d_8$ -**24**.<sup>182</sup>

Additionally, the reaction with enterolactone **52** was remarkably found to give fully aryl deuterated  $d_8$ -**52** (Scheme 24).<sup>183</sup> All eight aromatic protons, even from the non activated C-5 and C-5' sites, were exchanged at room temperature giving  $d_8$ -labelled product in over 99 % isotopic purity.  $d_{10}$ -**53** was prepared from  $d_8$ -**52** by reduction with  $LiAlD_4$ . No exchange was observed at the  $\alpha$ -carbonyl site.<sup>III</sup>



**Scheme 24** Reagents and conditions: a) i)  $D_2O$ /acetone, ii)  $D_3PO_4 \cdot BF_3/D_2O$ , rt, 20 h; b)  $LiAlD_4$ , THF, rt, 1 h; c)  $HClO_4$ , acetone.<sup>182,183</sup>

The reaction conditions of the reported acidic H/D exchange reactions for lignans (above and in the articles III and IV) as well as the yields and isotopic purities of the deuterated products are collected to Table 1 (p. 59) and discussed in Chapter 6.2.5.



## **5 The aims of the present study**

Our interest in lignans originated from the possible biological effects and health benefits of these compounds, and the work is part of an interdisciplinary research project entitled Lignans, Phytoestrogens and Human Health. As discussed in the previous sections, analytical and metabolic studies require deuterium labelled standards. Particularly, isomerically and isotopically pure stable polydeuterated lignans are needed in the development of quantitative analysis. To meet the overall goals, the aim of the present work was to examine the deuteration of lignans, i.e. how and in which order the H/D exchange occurs in a lignan framework, and what is the degree of the deuteration (i.e. isotopic purity). The experimental observations were compared to the results of computational studies (ESP charges or molecular modelling), and the further understanding on the deuteration reaction was utilised in synthesising stable and isotopically pure deuterium labelled lignans. Thus new, stable and isotopically pure deuterium labelled lignano-9,9'-lactones (**5**) and 9,9'-epoxylignanes (**6**) were synthesised, suitable for analytical studies as reference compounds.

Lignans have been recognised as interesting and challenging targets for organic synthesis due to their diverse structures as well as their important biological properties. Several synthetic approaches have been published over the years. The racemic syntheses are still under continuous development, but stereoselective syntheses are the predominant objective in this field, thus we also engaged in this challenge. While developing a general procedure for the stereoselective synthesis of 7'-hydroxylignano-9,9'-lactones, it was found that the stereoselective reduction of the 7' keto group with L-Selectride (Chapter 2.4) afforded a rearranged byproduct. The unexpected byproduct led to further studies on the rearrangement reactions in basic conditions. Thus aims of the present work were to examine the mechanism of the rearrangement of 7'-hydroxylignano-9,9'-lactones in basic conditions and to study the stereochemistry of the rearranged products. The absolute configurations of three lignan regio- and stereoisomers were proven by X-ray spectroscopy, and some conflicting or erroneous data in literature were clarified.

## 6 Results and discussion

### 6.1 Synthesis of lignans

Even though lignans are natural products, present in a number of various plants, synthetic methods are needed. With the exception of 7'-hydroxymatairesinol (**31**) and a few other examples,<sup>184</sup> the amount of lignans that is within one's reach from plant sources may be too small to even determine the structure of the molecule, not to mention any further studies. Thus access to sufficient amount of material is necessary.

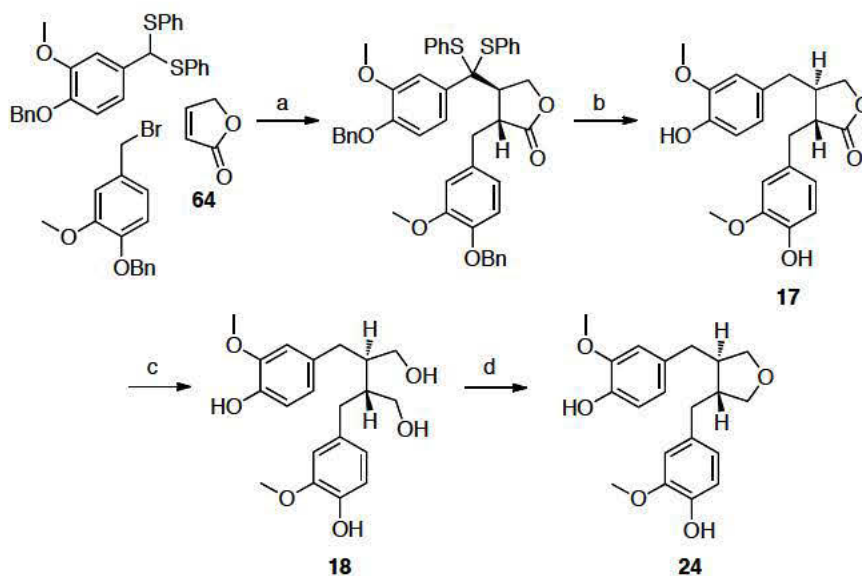
The lignans vary widely in structure. The lignano-9,9'-lactone skeleton is a synthetically important structure as it is a starting point for direct or indirect access to many other lignan types. Several methods for lignan synthesis have been published during the years, and from time to time they have been reviewed.<sup>3-7,22</sup> The methods usually applied in the synthesis of lignano-9,9'-lactones are Stobbe condensation and (tandem) conjugate addition reactions. The most recent syntheses of these structures are discussed in papers published in our laboratory.<sup>74,V</sup>

In our work we mainly concentrated on the lignano-9,9'-lactones and 9,9'-epoxylignanes. The lignans, needed as starting material for our deuteration experiments (Chapter 6.2 and original articles III and IV), were prepared starting with the tandem Michael addition-alkylation procedure (a in Scheme 25).<sup>185</sup> The use of the tandem conjugate addition reaction was first reported in the synthesis of a lignan skeleton by Damon *et al.*,<sup>186</sup> and has subsequently been extended to the synthesis of a wide variety of lignans by many research groups. The approach is dexterous in generating stereoselectively a *trans*-2,3-dibenzylbutyrolactone framework in one step.

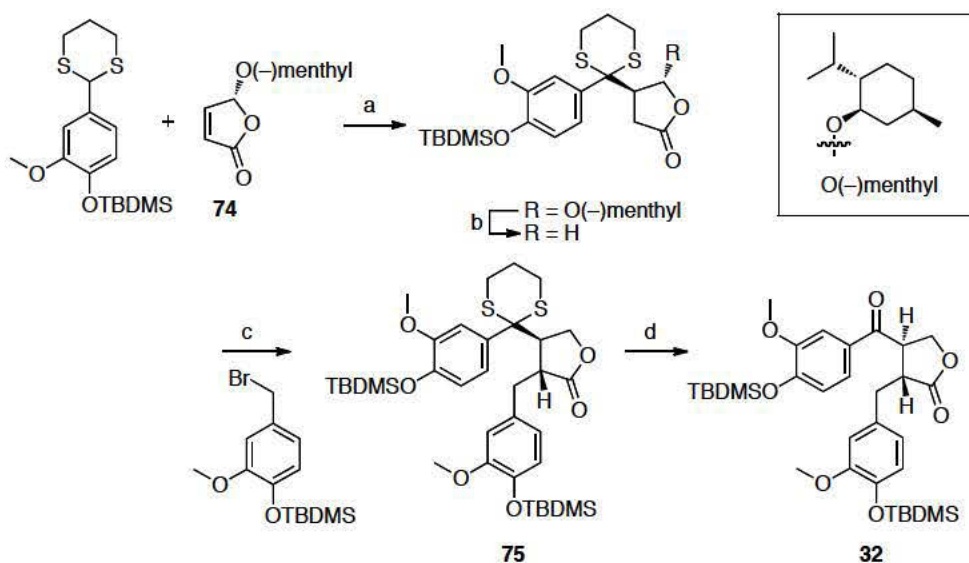
Raney nickel treatment of the benzyl protected dithioacetals (b in Scheme 25) provided both desulfurisation and simultaneous removal of the benzylic protections. The phenolic butyrolactone was used as such<sup>III</sup> or further reduced to the diol with LiAlH<sub>4</sub>. The 2,3-dibenzyl-1,4-butanediols were dehydrated to the 3,4-dibenzyltetrahydrofurans (9,9'-epoxylignanes) with conc. HCl under microwave irradiation at a low temperature in 30 minutes,<sup>V</sup> or used as such for the deuteration and simultaneous cyclisation procedure.<sup>IV</sup>

To study the rearrangement reactions of 7'-hydroxylignano-9,9'-lactones (Chapter 6.3 and original articles I and II), we synthesised the enantiopure 7'-oxolignan lactone starting materials using the Michael addition-alkylation procedure, thoroughly studied by Raffaelli *et al.* (Scheme 26).<sup>70</sup> The stereoselectivity of the tandem reaction was controlled by a chiral Michael acceptor, 5-(-)-menthyloxybutenolide **74**, which furnished the (8*R*,8'*R*)-enantiomer of the lignano-9,9'-lactone.





**Scheme 25** Synthesis of racemic 8,8'-*trans*-lignan skeleton, matairesinol **17**, secoisolariciresinol **18** and anhydrosecoisolariciresinol **24** as examples. Reagents and conditions: a) i) *n*-BuLi, THF,  $-78\text{ }^{\circ}\text{C}$ , ii) **64**, THF,  $-78\text{ }^{\circ}\text{C}$ ; iii) DMI, benzyl bromide, THF,  $-78\text{ }^{\circ}\text{C}$ ; b) Raney-Ni, EtOH, reflux; c)  $\text{LiAlH}_4$ , THF, d) conc. HCl, MW,  $40\text{ }^{\circ}\text{C}$ .<sup>185,V</sup>



**Scheme 26** Synthesis of enantiopure 7'-oxolignan lactones, silyl protected 7'-oxomatairesinol **32** as example. Reagents and conditions: a) *n*-BuLi, THF, DMPU,  $-78\text{ }^{\circ}\text{C}$ ; b) i)  $\text{NaBH}_4$ , KOH, EtOH,  $0\text{ }^{\circ}\text{C}$ , then rt; ii)  $0.1\text{ N HCl}$  (pH 5–6); c) LDA or LHMDS, THF, DMI,  $-78\text{ }^{\circ}\text{C}$  to rt; d)  $(\text{CF}_3\text{COO})_2\text{IPh}$ ,  $\text{CH}_3\text{CN}-\text{H}_2\text{O}$ , rt.<sup>70</sup>

## 6.2 Synthesis of deuterium labelled lignans

### 6.2.1 Synthesis of new deuterium labelled lignano-9,9'-lactones using $D_3PO_4 \cdot BF_3/D_2O$

In our laboratory, the deuteration reagent  $D_3PO_4 \cdot BF_3/D_2O$  was found to be very powerful in labelling isoflavones and especially lignans, exchanging protons to deuteriums even at the unactivated aromatic positions of the lignan skeleton ( $d_8$ -**52** in Scheme 24). Thus the reagent was taken under further studies and seven new deuterium labelled lignanolactones were synthesised (Figure 11).<sup>187,III</sup> A while earlier the metabolism of the plant lignans syringaresinol, medioresinol, secoisolariciresinol and matairesinol had been studied.<sup>98,188</sup> In addition to enterolactone and enterodiol several other metabolites were detected but they remained unidentified due to the lack of authentic reference compounds. Based on the mass spectra several possible structures were postulated, and they were presumed to possess dibenzylbutyrolactone or dibenzylbutanediol type structures with variation concerning the presence of hydroxy and methoxy groups and their positions on the aromatic rings. Therefore variously substituted lignano-9,9'-lactones possessing the structures of the postulated metabolites were synthesised.<sup>185</sup> Three of them (**76–78**) were taken to deuteration experiments, and four new structures (**79–82**) were synthesised to study further the influence of the substitution pattern on the deuteration order.

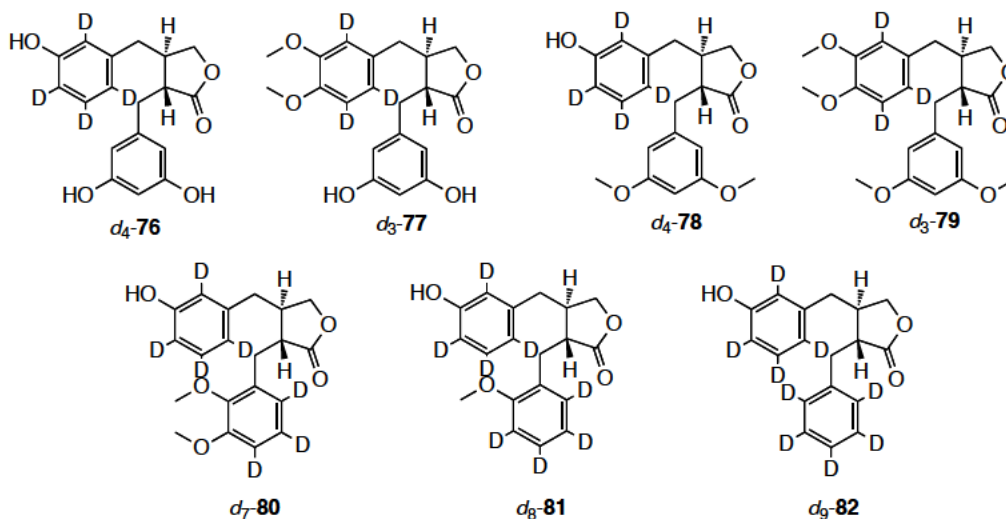
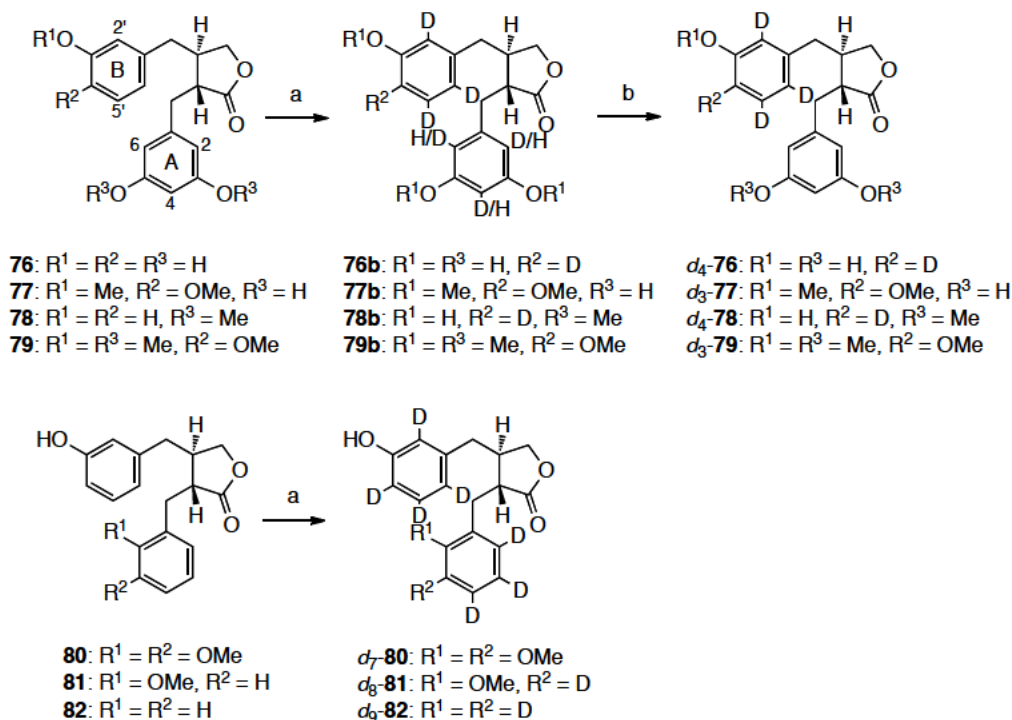


Figure 11 New deuterium labelled lignans.<sup>III</sup>

The deuteration reagent  $D_3PO_4 \cdot BF_3/D_2O$  is freshly prepared by dissolving dry  $P_2O_5$  in  $D_2O$  at 0 °C, and the resulting deuterated phosphoric acid is saturated with  $BF_3$  gas bubbling through the acid at room temperature. When this reagent was used for the isoflavones daidzein and dihydrodaidzein, the degree of deuteration could be modulated by the reaction temperature.<sup>178,180</sup> In the case

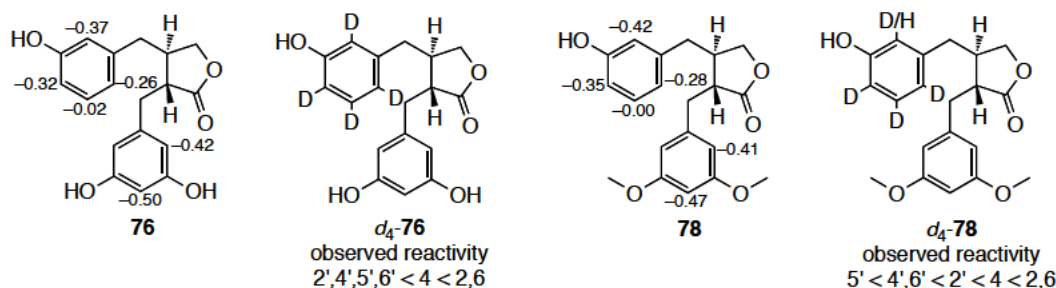
of isoflavonoids<sup>178</sup> and flavones,<sup>179</sup> rising the temperature up to 55 °C does not seem to diminish the yields, but the lignan framework was found to be more fragile. Thus the deuteration reactions were carried out at room temperature (Scheme 27). It is also interesting that within the lignan skeleton, all the aromatic sites underwent exchange to deuteriums at room temperature, while in the case of the isoflavone daidzein, only three positions are deuterated at room temperature in three days, while repeating the reaction deuterates the fourth position and rising the temperature up to 55 °C deuterates the fifth position.<sup>178</sup>



**Scheme 27** Synthesis of stable deuterated lignano-9,9'-lactones. Reagents and conditions: a)  $D_3PO_4 \cdot BF_3 / D_2O$  (freshly prepared), rt; b)  $CH_3COCl$ , MeOH, reflux.<sup>iii</sup>

The exchange order of hydrogens, i.e. the relative ease of H/D exchange at the various aromatic positions, was determined by following the progress of the deuteration reaction by  $^1H$ -NMR. In addition, electrostatic potential (ESP) charges were calculated to study the relative reactivities of the aromatic protons. The observed reactivities were compared with the calculated reactivities, and they were found to be in a good agreement. The very inactive 5-position in the 3-hydroxyphenyl moiety (5' in **76**, **78**, **80–82**, Scheme 27) was tardy in reacting and in some cases needed several repetitions with fresh deuteration reagent. On the other hand, two *meta*-substituted hydroxy or methoxy groups in ring A of **76–79** highly activated the aromatic sites 2, 4 and 6. Because of the strong activation at these sites (2, 4 and 6), back exchange from deuterium to hydrogen occurred in some extent during quenching the deuteration reaction into large volume of ice cold water (0.5 l water/100 mg of

lignan). Thus, when the deuteration had reached a satisfactory level, i.e. the isotopic purity was more than 85 %, the remaining labile deuterium labels were selectively back exchanged to hydrogens in refluxing 0.5–1 % CH<sub>3</sub>COCl in methanol (Scheme 27). The deuteriums in the ring B of **76**, **77** and **79** were not affected, neither were any deuteriums in lignans **80–82** (which were also refluxed for 30 minutes in 0.5 % CH<sub>3</sub>COCl/MeOH). However, the 2'-position in **78** was more prone to back exchange giving a mixture of *d*<sub>4</sub>- and *d*<sub>3</sub>-products in 85:15 ratio (Figure 12).



**Figure 12** Calculated ESP charges for unsubstituted aromatic positions in molecules **76** and **78**. Smaller ESP value indicates a lower electron charge density and thus, in this case, more labile protons in the aromatic ring. Hence the ESP charges reveal that the order of reactivity in electrophilic substitution for compound **76** is 5' < 4' < 6' < 2' < 2,6 < 4, and for compound **78** 5' < 4' < 6' < 2' < 2,6 < 4. The observed reactivity was in good agreement with the calculated ones. The discrepancy between calculated and observed reactivity in the cases of position 4 may be due to steric reasons.<sup>iii</sup>

In all the method, deuteration with D<sub>3</sub>PO<sub>4</sub>·BF<sub>3</sub>/D<sub>2</sub>O and removing the labile deuteriums in CH<sub>3</sub>COCl/MeOH, was proved to be very efficient in synthesising labelled lignans possessing three to nine deuteriums in the aromatic rings. The deuterated lignans were isomerically and isotopically pure (> 85 % isotopic purity) and the yields were good (81–97 %).

### 6.2.2 Synthesis of deuterium labelled lignano-9,9'-lactones and 9,9'-epoxylignanes using DCl/D<sub>2</sub>O under microwave heating

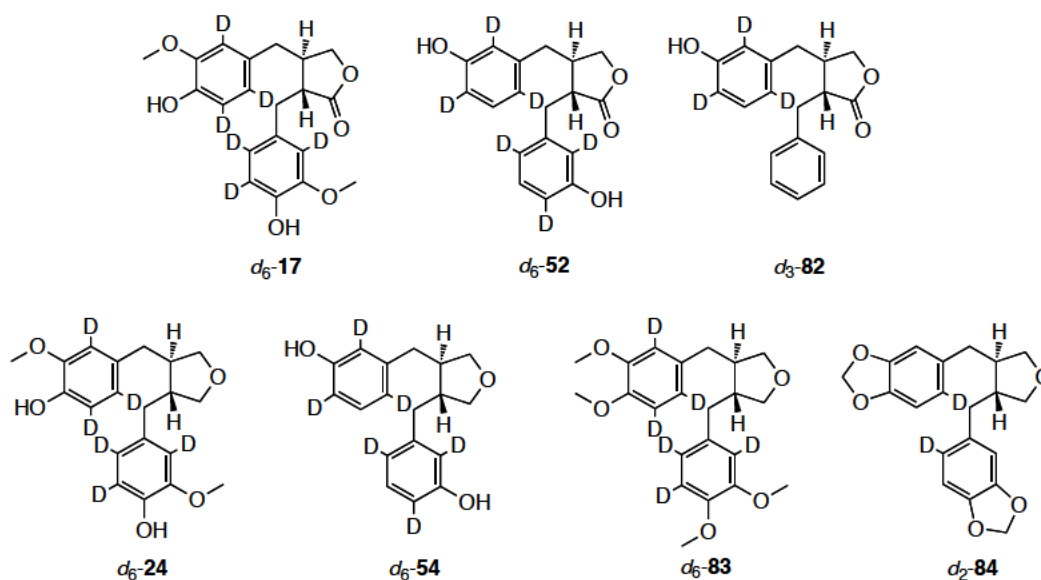
Although the deuteration method with a deuterated phosphoric acid–boron trifluoride complex (D<sub>3</sub>PO<sub>4</sub>·BF<sub>3</sub>/D<sub>2</sub>O) worked fine in labelling lignans, offering good yields and high isotopic purity, the reaction times were quite long partly due to the poor solubility of lignans in water.

The use of dielectric microwave heating has been utilized in organic synthesis at an increasing rate since the pioneer work of Gedye<sup>189</sup> and Giguere<sup>190</sup> in 1986. In spite of having become in frequent and common use in laboratory work, microwave techniques have not been reported in the synthesis nor labelling of lignans until very recently.<sup>191,192,193</sup> In our laboratory it was found that using 35 % DCl in D<sub>2</sub>O and the ionic liquid, 1-butyl-3-methylimidazolium chloride, [bmim]Cl as a cosolvent under microwave



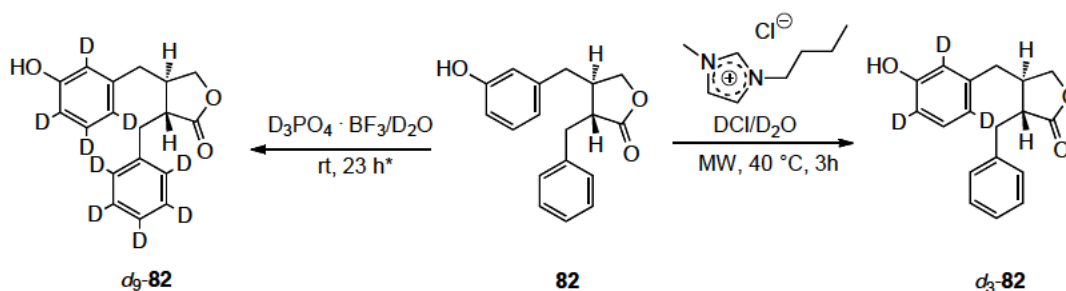
irradiation, is a fast and high-yielding deuteration method for isoflavonoids and lignans.<sup>191,194</sup> The reaction times were shortened significantly.

Lignans have been shown to be susceptible towards heating. The method using DCl/D<sub>2</sub>O in MW works well for lignans even at lower temperatures (40 °C) and in some cases also without the dissolving help of an ionic liquid (Figure 13 and Scheme 28).<sup>IV</sup>



**Figure 13** Deuterolabelled lignans. *d*<sub>3</sub>-**82**, hexadeuterated enterofuran *d*<sub>6</sub>-**54** and brassilignan *d*<sub>6</sub>-**83** and dideuterated dehydroxycubebin *d*<sub>2</sub>-**84** are new compounds.<sup>IV</sup>

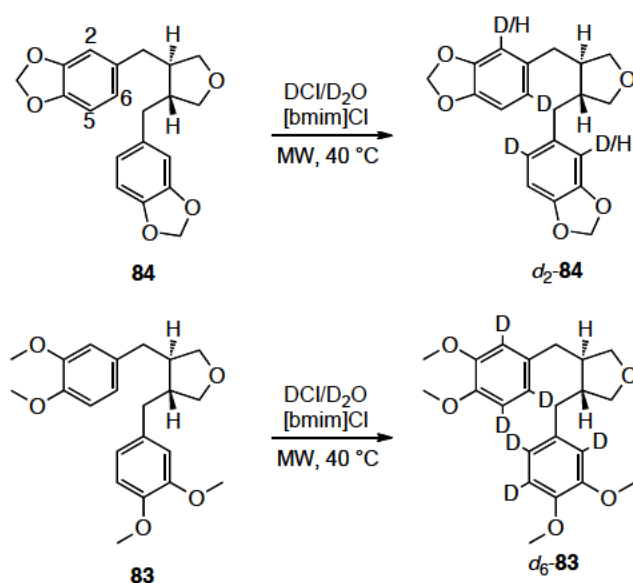
Deuteration using the highly acidic D<sub>3</sub>PO<sub>4</sub>·BF<sub>3</sub>/D<sub>2</sub>O, which exchanges protons to deuteriums also at the less reactive aromatic sites, gave a fully aryl deuterated derivative of **82** (with nine deuteriums, *d*<sub>9</sub>-**82**).<sup>III</sup> The DCl based method worked in a more conventional way, exchanging protons at the substituted B-ring only and there at the activated sites *ortho* and *para* to the hydroxy group (Scheme 28).<sup>IV</sup>



**Scheme 28** Deuteration of 3-hydroxylignano-9,9'-lactone **82**.<sup>III,IV</sup> \*The deuteration reaction was repeated with fresh reagent until the isotopic purity was more than 85 %.

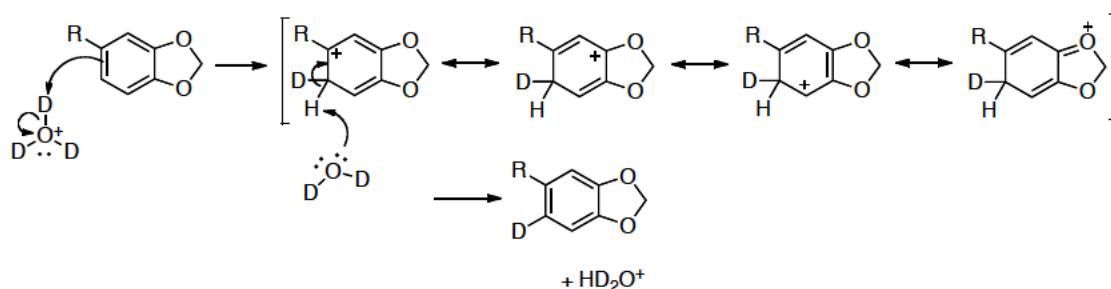
### 6.2.3 Deuteration of dehydroxycubebin

When deutrating with DCl in D<sub>2</sub>O, all the other lignan structures behaved as expected, exchanging protons to deuteriums at all the activated positions (Figure 13), but dehydroxycubebin **84** gave unexpected results (Scheme 29).<sup>iv</sup> Even after repeating the reaction with fresh deuteration reagents and recycling the isolated crude product *d*<sub>2</sub>-**84** the deuteration was left partial. Quantitative <sup>13</sup>C NMR studies demonstrated that the 6- and 6'-positions were fully deuterated, while only about a third of the 2- and 2'-sites carried deuteriums and the positions 5 and 5' had remained intact. According to EI-MS there was a mixture of di-, tri- and tetra-deuterated products. However, brassilignan **83**, a close analog of dehydroxycubebin, was successfully hexadeuterated with over 97 % isotopic purity.



**Scheme 29** Deuteration of dehydroxycubebin **84** and brassilignan **83**.

The mechanism of the H/D exchange is an ordinary electrophilic aromatic substitution,<sup>172</sup> taking place in two steps: first the electrophile attacks giving rise to positively charged resonance stabilised intermediate, called an arenium ion, Wheland intermediate or sigma complex, and the leaving group departs in the second step (Scheme 30). Simultaneous attack and departure mechanisms are not known.<sup>175</sup> The degree of deuterium incorporation should be governed by equilibrium considerations of the participant D and H species present in D<sub>2</sub>O and at the reactive aryl sites.



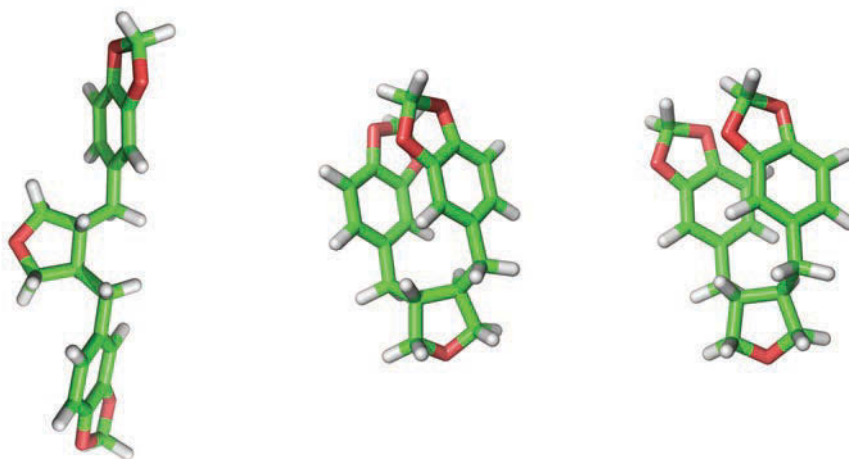
**Scheme 30** The mechanism of H/D exchange. R is for the rest of the lignan molecule.

Computational studies and additional experiments with model compounds were performed to understand the differences between the two close analogues and clarify the observed discrepancy in the reactivity of dehydroxycubebin **84**.<sup>iv</sup> Deuteration experiments with a model compound 1,3-benzodioxole (1,2-methylenedioxybenzene, Scheme 30: R=H) gave results similar to **84**: the positions that were *meta/para* to the methylenedioxy substituent were fully deuterated, while the *ortho* positions were deuterated in part only. All the aromatic positions of the model compound veratrole (1,2-dimethoxybenzene), however, were fully deuterated.

The higher *para*-selectivity of 1,3-benzodioxole in electrophilic aromatic substitution, in comparison to its cyclohomologues or veratrole, has been observed and rationalised with distortion of the aromatic ring when annelated to a small ring (so-called Mills–Nixon effects<sup>195,196</sup>) or a quasiaromatic nature of the heterocyclic ring of 1,3-benzodioxole.<sup>197</sup> Sesamol (3,4-methylenedioxyphenol, Scheme 30: R=OH) and its ethers are also known to undergo a rapid acid-catalysed exchange of H-6 but a slower exchange of H-2.<sup>198</sup> Interestingly, deviant behaviour of 1,3-benzodioxole has been recently reported in certain other types of reactions as well.<sup>199</sup>

The 9,9'-epoxylignanes can adopt three main conformations (Figure 14). The calculations implied that the  $\pi$ -stacked, so called sandwich conformations are preferred under the reaction conditions.





**Figure 14** Optimised structures for the three different conformations of dehydroxycubebin **84**: extended (on the left) and two  $\pi$ -stacked conformations. The stacked conformations are preferred under the deuteration reaction conditions, and the stacked structure in the middle is energetically slightly lower than the stacked conformation on the right, probably due to more favorable multipole interactions between the oxygen containing substituents.

As in the deuteration mechanism, the deuteration reaction path was broken into two steps also in the computational studies: addition of a proton from one face of the ring plane, and abstraction of a proton from the other face. The reaction from both faces is illustrated in Figure 15, where the face of the ring plane exposed to the solvent is denoted “up” and the other face is “down”. The difference in the activation energies suggested that the deuterium is added from the “up” face, where the steric pressure is weaker, and the proton leaves from the “down” face.



**Figure 15** The “up” and “down” proton addition illustrated in the dehydroxycubebin molecule.

The calculations suggested that deuterium can be added to the 5-position of dehydroxycubebin **84** from the “up” face of the molecule, but the transition state with the water molecules approaching from the “down” face was not feasible. Therefore the proton could not leave and the second step of the electrophilic aromatic substitution reaction could not occur. Instead, the

reaction is reversed and the deuterium is liberated back to the solution. The 2-position differs slightly from the 5-position in being less restricted and is partially deuterated.

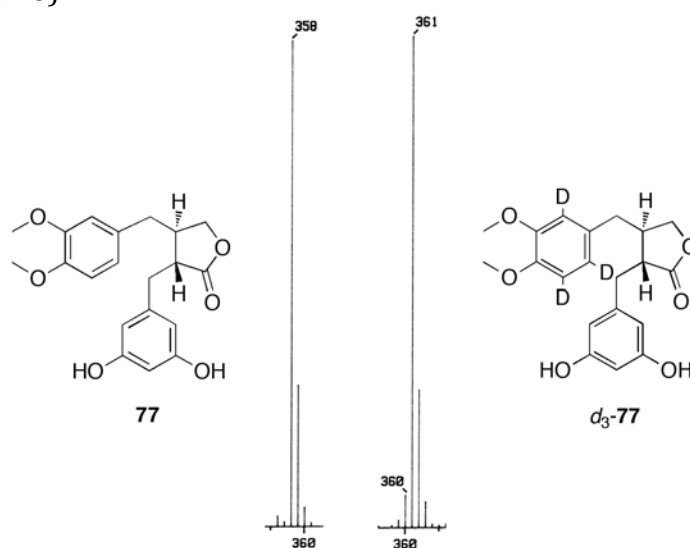
Brassilignan **83**, whose methoxy groups are flexible and freely rotating, can accommodate the water molecules on the “down” face, and both reaction steps are possible.

The computational structural analysis together with the deuteration results indicate that the aryl rings cannot rotate freely in either of the lignan molecules. Thus there are two atropisomers, i.e. the stacked conformers, and the molecules do not switch between them. It seems that there are two factors preventing the deuteration of being completed in positions 2 and 5 in dehydroxycubebin **84**: one conformation-independent, probably quasiaromatic nature of the five-membered methylenedioxy substituent ring, that restricts the complete deuteration at *ortho*-positions. The other effect is steric, conformation dependent, and blocks even the partial deuteration at the C-5 (and C-5') of **84**.

#### 6.2.4 Characterisation of the deuterated compounds

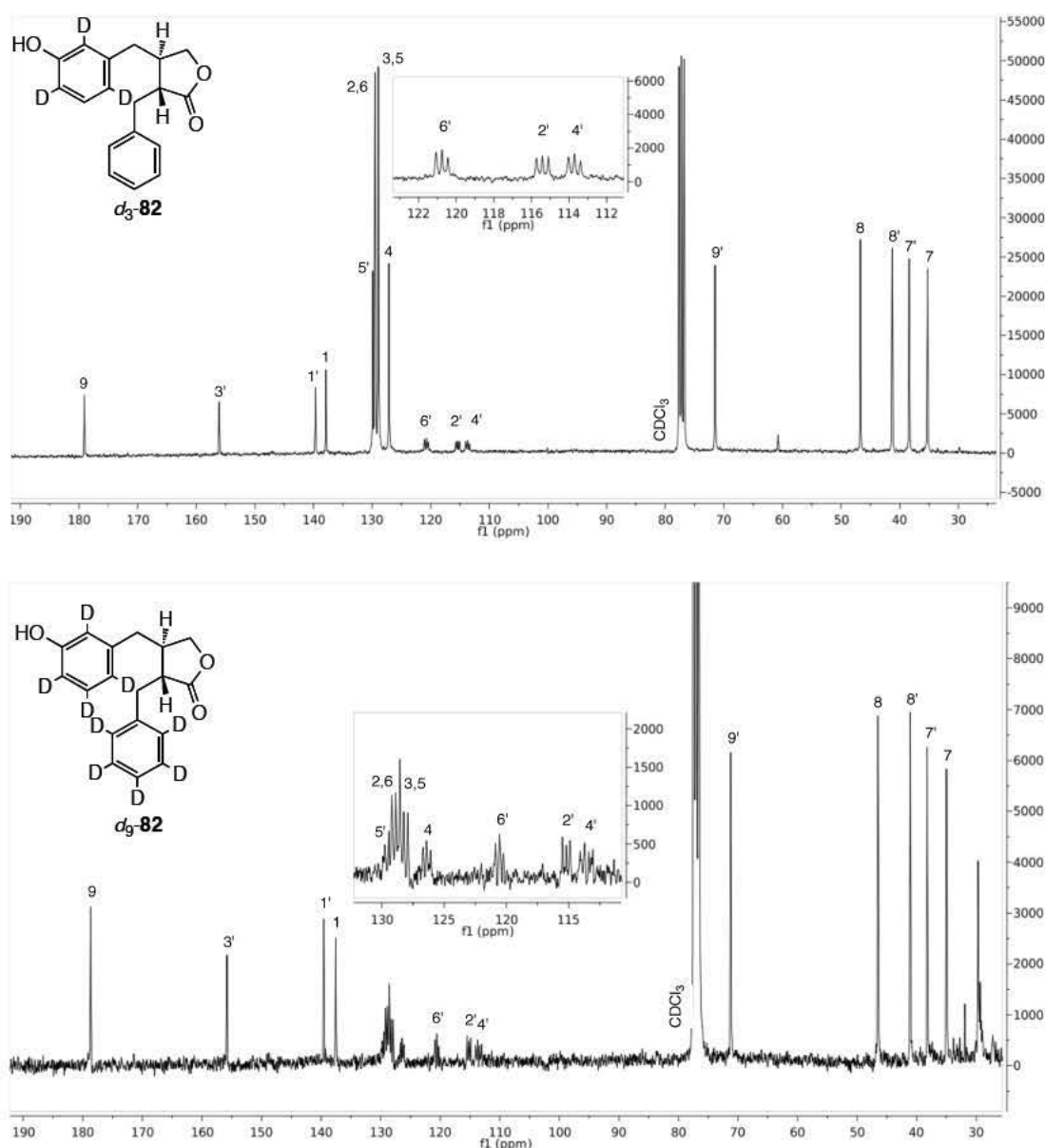
The deuterated compounds were characterised by NMR and MS and by comparison of the spectra with those of undeuterated analogues. The number of deuterium atoms in a product as well as the isotopic purity was determined by MS (EI or ESI). The locations of the deuterium atoms were established from  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR studies.

The isotopic purities of deuterated lignans in this study were determined from the ion clusters in the molecular ion region in the EI or ESI mass spectra by comparison with those of undeuterated compounds. For the native butyrolactone and tetrahydrofuran type lignans the M-1 and M-2 peaks usually are very small (2–5 % of the intensity of the molecular ion peak) or totally absent (Figure 16).

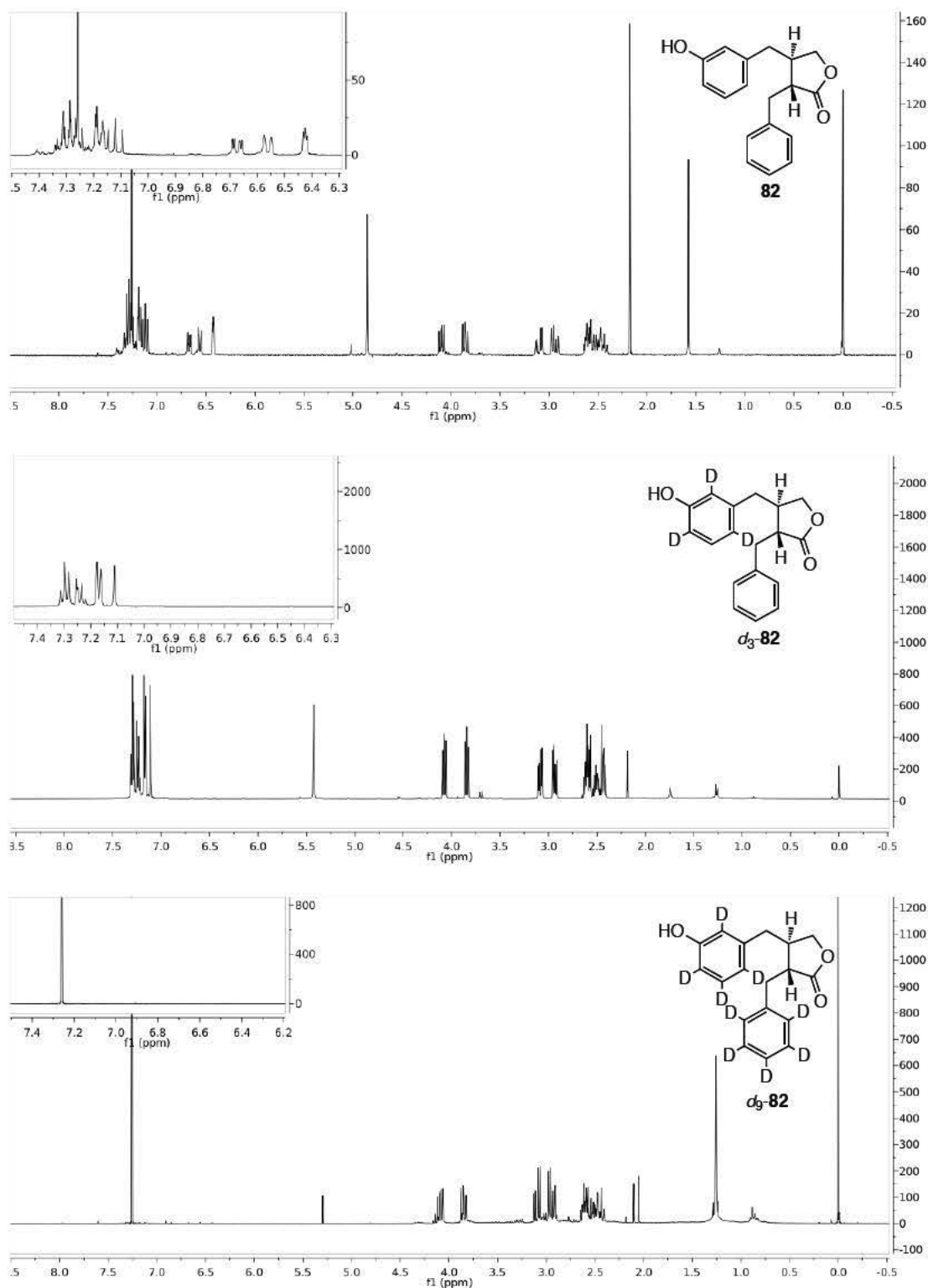


**Figure 16** The molecular ion region of unlabelled **77** ( $M = 358$ ) and stable deuterium labelled analog  $d_3$ -**77** ( $M = 361$ ).

In the  $^{13}\text{C}$ -NMR spectrum, the carbon atom bearing a deuterium atom produces a low-intensity triplet (instead of the fairly intensive aromatic C–H singlet) since the spin of deuterium is 1 (Figure 17). The  $^1\text{H}$ -NMR spectra of deuterium labelled compounds are the same as for unlabelled compounds, with the exception that the aromatic deuterons can not be seen and the coupling pattern of the protons attached to the adjacent carbons are simplified (Figure 18).



**Figure 17**  $^{13}\text{C}$ -NMR spectra of  $d_3$ - and  $d_9$ -labelled 3'-hydroxylignano-9,9'-lactones  $d_3$ -82<sup>IV</sup> and  $d_9$ -82<sup>III</sup> (in  $\text{CDCl}_3$ ). The C–H singlets for deuterated sites are replaced by low intensity C–D triplets (expanded).



**Figure 18**  $^1\text{H}$ -NMR spectra of unlabelled,  $d_3$ -labelled<sup>IV</sup> and  $d_9$ -labelled<sup>III</sup> 3'-hydroxylignano-9,9'-lactone **82** (in  $\text{CDCl}_3$ ). In the expanded aromatic region it is clearly seen, that proton signals are lacking when the aromatic positions are deuterated.

### 6.2.5 H/D exchange reactions for lignans

The results in deuterating lignans, in terms of isotopic purity, are rather good regardless of the acidic deuteration reagent used. The first H/D exchange reaction was done for enterolactone **52** with refluxing DBr in D<sub>2</sub>O (Scheme 21, p. 43), which gave relatively poor yield, only 35 %, although the isotopic purity was over 90 %. Probably the lignan molecule did not fully survive the refluxing conditions, and since then good yields and isotopic purities have been achieved at lower temperatures.

Comparing the reaction times (Table 1), it seems that long reaction times result from solubility issues: using any dissolving help, such as THF/CDCl<sub>3</sub> (entry 3) or an ionic liquid [bmim]Cl (entry 5), shortens the reaction times significantly. Although some lignans, e.g. matairesinol, were able to be deuterated in DCl/D<sub>2</sub>O in 30 minutes without the help of ionic liquid, most of them gained from presence of a co-solvent. Attempts to deuterate enterolactone **52** in DCl/D<sub>2</sub>O without a co-solvent resulted a ca 1:1 mixture of unchanged starting material *d*<sub>0</sub>-**52** and hexadeuterated enterolactone *d*<sub>6</sub>-**52**. When deuterioethanol (CH<sub>3</sub>CH<sub>2</sub>OD) was used as a co-solvent, no unlabelled species were present, but reaching *d*<sub>6</sub>-**52** in 85 % isotopic purity needed longer reaction times (4 hours, entry 6) than with ionic liquid [bmim]Cl (40 min, entry 5).

**Table 1.** Reaction conditions of known acidic H/D exchange reactions for lignans.

entry	acid	pK <sub>a</sub> *	solvent	co-solvent	product	T	t	yield (%)	ip (%)	ref.
1	DBr	-9	D <sub>2</sub> O	–	<i>d</i> <sub>6</sub> - <b>52</b>	reflux	5 h	35	>90	176
2	D <sub>3</sub> PO <sub>4</sub>	2.12	D <sub>2</sub> O	–	<i>d</i> <sub>6</sub> - <b>17</b>	80 °C	3x3 days	82	95	138
3	DClO <sub>4</sub>	-10	THF/ CDCl <sub>3</sub> (1:1)	D <sub>2</sub> O	<i>d</i> <sub>6</sub> - <b>17</b>	rt	4 h	84	**	166
4	D <sub>3</sub> PO <sub>4</sub> ·BF <sub>3</sub> in D <sub>2</sub> O	***	D <sub>2</sub> O	–	several	rt	20 h to several days	81–97	85–99	183 III
5	DCl	-8	D <sub>2</sub> O	[bmim]Cl	several	40 °C	30–120 min	74–84	85–97	IV
6	DCl	-8	D <sub>2</sub> O	CH <sub>3</sub> CH <sub>2</sub> OD	<i>d</i> <sub>6</sub> - <b>52</b>	40 °C	4 h		85	****

\* pK<sub>a</sub> of the corresponding protic acid.<sup>200</sup>

\*\* Not mentioned.

\*\*\* The exact structure is unknown, but the reagent is stated to be highly acidic.<sup>181</sup>

\*\*\*\* Unpublished result.

The corresponding protic acids related to DBr, DCl and DClO<sub>4</sub> are all strong Brønsted-Lowry acids, having pK<sub>a</sub>-values -8 or lower. D<sub>3</sub>PO<sub>4</sub>·BF<sub>3</sub>/D<sub>2</sub>O is reported to be highly acidic.<sup>181</sup> Greenwood and Thompson have proposed a structure for the anhydrous complex,<sup>201</sup> yet the exact structure or the pK<sub>a</sub> value of the aqueous complex are not known. However, it has a remarkable ability to exchange protons even at the highly unactivated positions. The fairly long reaction times (20 hours and more) probably result from poor solubility.

Our acidic deuteration methods have proved to work for several variously substituted tetrahydrofuran and lactone type lignans affording stable and isotopically pure polydeuterated products in good yields. When choosing a

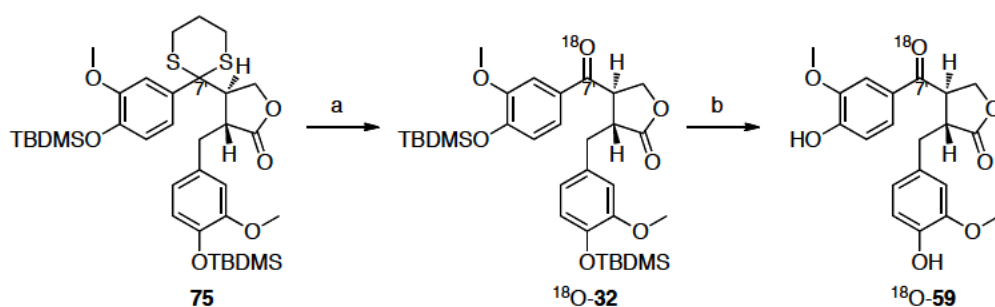
suitable method to deuterate a lignan, one has to take into account the intended use of the labelled compound and its aromatic substitution pattern. If the labelled compound is aimed as an internal standard, three to five labels are considered optimal. If there are highly activated sites, such as in a resorcinol type substitution (e.g. in **76**, p. 51), the labile deuteriums must be back exchanged, and in such a case it may be necessary to make use also of the possible inactive aromatic sites to obtain stable labels in sufficient numbers. The deuteration reagent  $D_3PO_4 \cdot BF_3 / D_2O$  would be a good choice then. However, if the substitution pattern provides enough sites for stable labels, the more conventional conditions, such as the commercially available  $DCl / D_2O$  with a co-solvent, is a practical option. In short, the suitable method is case-specific.

### 6.3 Rearrangement and stereochemistry studies

As mentioned in the literature review of 9'-hydroxylignano-9,7'-lactones (Chapter 2.4), the many possible rearrangement mechanisms of 7'-hydroxylignano-9,9'-lactones and also the stereochemistry of the products were not completely clarified. Therefore we wanted to investigate the three mechanisms that are discussed in the article of Raffaelli *et al.*<sup>70</sup> The mechanistic studies were carried out using  $^{18}O$  labelled compounds and monitoring the results by mass spectrometry. Also different rearrangement conditions were investigated with various unlabelled compounds.

#### 6.3.1 Translactonisation studies with oxygen-18 labelled compounds

For the mechanistic studies, three compounds having  $^{18}O$  labels at the C-7' were synthesised: silyl protected 7'-oxomatairesinol  $^{18}O$ -**32**, 7'-oxomatairesinol  $^{18}O$ -**59** and acetylparabenzlactone (7'S)- $^{18}O$ -**37**. The  $^{18}O$  label was introduced during the deprotection of the dithiane moiety of **75** using  $^{18}OH_2$  as cosolvent (Scheme 31).<sup>1</sup>



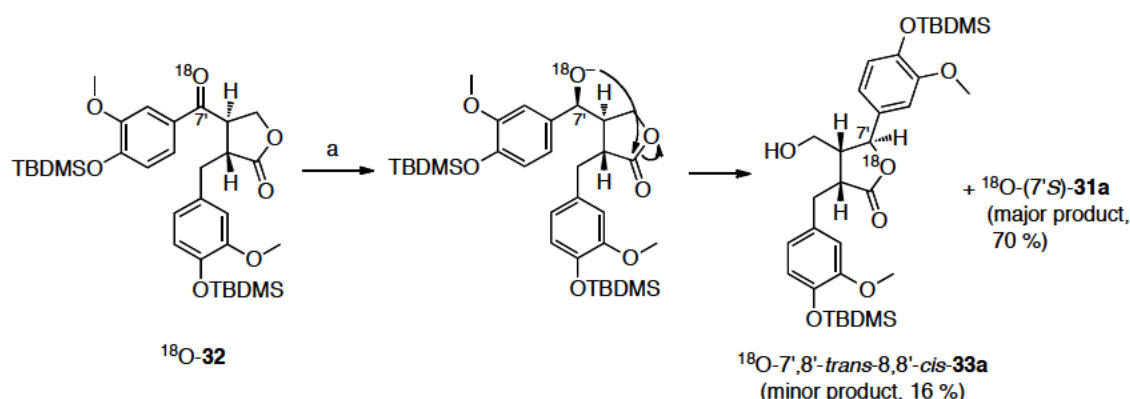
**Scheme 31** Synthesis of oxygen-18 labelled lignans **32** and **59**. Reagents and conditions: a)  $(CF_3CO_2)_2IPh$ ,  $^{18}OH_2$ ,  $CH_3CN$ , rt; b) TBAF, AcOH, THF, 0 °C.<sup>1</sup>

The mechanistic studies were planned as follows. 1) L-Selectride reduction of the silyl protected  $^{18}O$ -7'-oxomatairesinol to give the major product,  $^{18}O$ -(7'S)-

hydroxymatairesinol ( $^{18}\text{O}$ -(7'*S*)-**31a**) and rearranged byproduct. If the byproduct was formed via translactonisation, the  $^{18}\text{O}$  label would remain in the product (see the proposed mechanism in Scheme 6, p. 22). 2) L-Selectride reduction of  $^{18}\text{O}$ -7'-oxomatairesinol with free phenols to give the major major product, (7'*S*)-hydroxymatairesinol and the rearranged byproduct. If the rearrangement occurred via a *para*-quinone methide intermediate (Scheme 5, p. 21), the  $^{18}\text{O}$  label would be cleaved from the rearranged byproduct. 3) The treatment of  $^{18}\text{O}$ -(7'*S*)-acetylparabenzlactone ( $^{18}\text{O}$ -(7'*S*)-**37**) with KOH/MeOH to give the  $^{18}\text{O}$ -(7'*S*)-parabenzlactone ( $^{18}\text{O}$ -(7'*S*)-**29a**) and a rearranged byproduct **36a**. As discussed in the Chapter 2.4, Niwa *et al.* had suggested the rearranged lactone to possess the all-*cis* stereochemistry,<sup>65</sup> which would result from an  $\text{S}_{\text{N}}2$  mechanism<sup>70</sup> (Scheme 7, p. 22) detaching the  $^{18}\text{O}$  label.

### 6.3.1.1 Translactonisation

The 7'-oxomatairesinol  $^{18}\text{O}$ -**32** was treated with L-Selectride (Scheme 32). If the rearrangement mechanism for silyl protected 7'-oxomatairesinol **32** was a translactonisation, the  $^{18}\text{O}$  label would remain in the product. The mass spectra showed that the  $^{18}\text{O}$  label was totally conserved, thus verifying the proposed translactonisation mechanism and the 7',8'-*trans*-8,8'-*cis*-stereochemistry as well.



**Scheme 32** L-Selectride reduction of silyl protected 7'-oxomatairesinol 7'- $^{18}\text{O}$ -**32**. Translactonisation mechanism conserves the oxygen-18 label in the products.<sup>1</sup> Reagents and conditions: a) L-Selectride, THF,  $-78^{\circ}\text{C}$ .

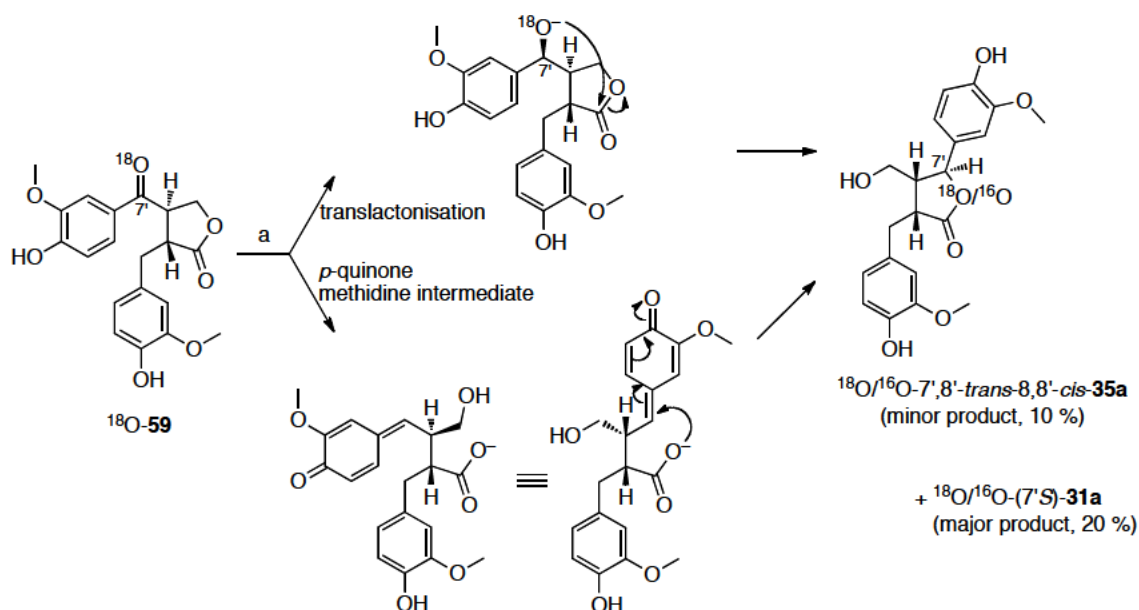
### 6.3.1.2 *para*-Quinone methide intermediate

For 7'-oxomatairesinol  $^{18}\text{O}$ -**59** we had to find an alternative way to Eklund's *et al.* original procedure,<sup>76</sup> since their yield of the rearranged product was low (5 % for the major byproduct, see Scheme 5, p. 21) and we could synthesise only a few dozen milligrams of the labelled lactone due to the high cost of oxygen-18 labelled water. We figured that since our rearranged lactone 7',8'-*trans*-8,8'-*cis*-**33a** from the L-Selectride reduction of **32** had the same



stereochemistry than 7',8'-*trans*-8,8'-*cis*-**35a**, the major product from Eklund's *et al.* procedure, the *p*-quinone methide mechanism could be demonstrated also with L-Selectride as a base (Scheme 33).

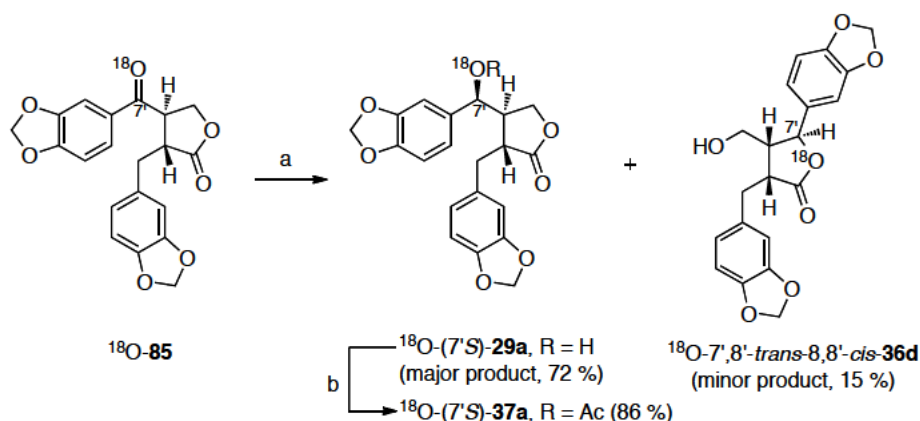
According to the MS, only part of the oxygen-18 label was lost, and if *p*-quinone methide was the only mechanism, all the oxygen-18 would have been lost. Thus in our case there probably are two parallel mechanisms: translactonisation ( $^{18}\text{O}$  conserved) and *p*-quinone methide mechanisms ( $^{18}\text{O}$  displaced by  $^{16}\text{O}$ ).



**Scheme 33** L-Selectride reduction of 7'-oxomatairesinol  $^{18}\text{O}$ -**59**. Some of the oxygen-18 labels were lost due to the *p*-quinone methide mechanism. Reagents and conditions: a) L-Selectride, THF,  $-78^\circ\text{C}$ .

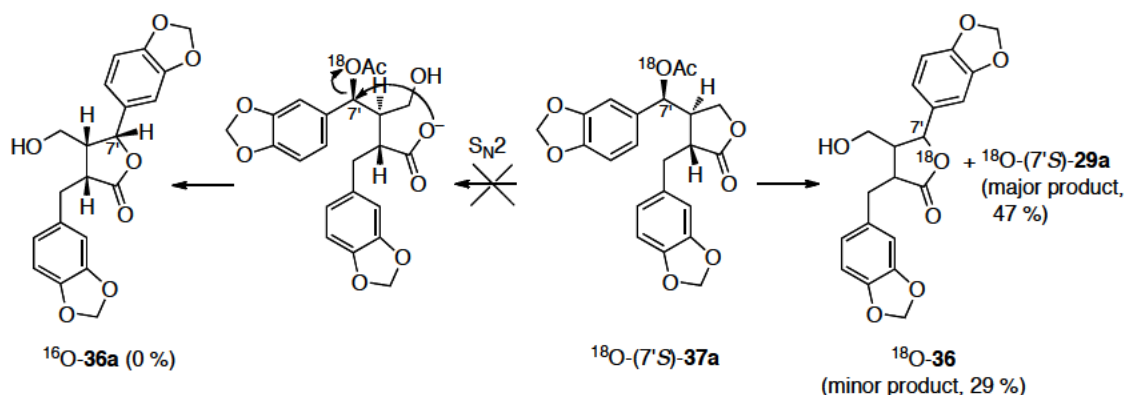
### 6.3.1.3 $S_N2$ mechanism

The oxygen-18 labelled acetylparabenzlactone  $^{18}\text{O}$ -(7'*S*)-**37a** was synthesised from the 7'-oxoparabenzlactone  $^{18}\text{O}$ -**85**, and the oxygen-18 label was conserved through all the reaction steps (Scheme 34). In the L-Selectride reduction also the translactonised byproduct was now detected,<sup>I,II</sup> contrary to the previous experiment.<sup>70</sup>



**Scheme 34** L-Selectride reduction of 7'-oxoparabenzlactone  $^{18}\text{O}-85$ . A new compound  $^{18}\text{O}-7',8'\text{-trans-}8,8'\text{-cis-}36\text{d}$  was also detected. Reagents and conditions: a) L-Selectride, THF,  $-78^\circ\text{C}$ ; b)  $\text{Ac}_2\text{O-Py}$ , rt.<sup>1,11</sup>

The acetylparabenzlactone  $^{18}\text{O}-(7'S)\text{-}37\text{a}$  was then treated with 2 % KOH in MeOH to repeat Niwa's *et al.*<sup>65</sup> procedure (Scheme 35). If the reported byproduct was formed via an  $\text{S}_{\text{N}}2$  mechanism as proposed (Scheme 7, p. 22),<sup>70</sup> the oxygen-18 label would have been lost. The two products were separated by flash chromatography, and contrary to our expectations the MS of the rearranged product showed conservation of the oxygen-18 label, and therefore the mechanism could not be  $\text{S}_{\text{N}}2$ .



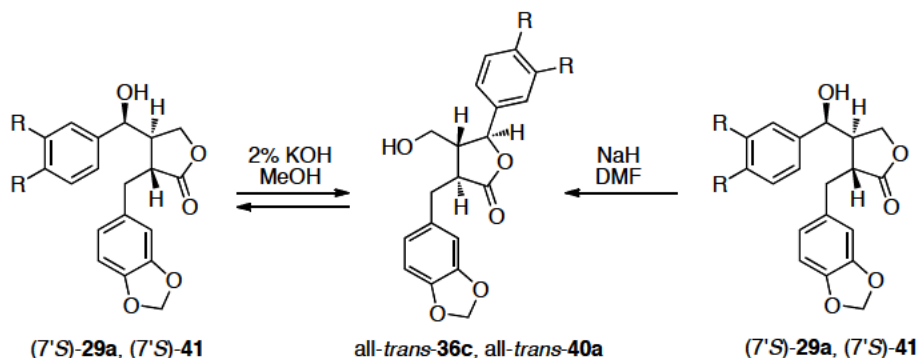
**Scheme 35** Treatment with 2% KOH in MeOH retained the oxygen-18 label in the rearranged product. Therefore the mechanism could not be  $\text{S}_{\text{N}}2$ .

Because of these results we started to re-examine the stereochemistry of the rearranged lactone **36**, since it was obvious that it could not be an all-*cis* structure, even if its NMR data ( $7'\text{H}$   $\delta$  5.13 ppm,  $J_{\text{H-}7',\text{H-}8'} = 8.5$  Hz) had some resemblance to Eklund's minor product all-*cis-}35\text{b} ( $7'\text{H}$   $\delta$  5.12 ppm,  $J_{\text{H-}7',\text{H-}8'} = 9.3$  Hz). We thoroughly compared the published NMR data and realised that the reported all-*trans* products from Moritani *et al.*<sup>71</sup> (all-*trans-}40\text{a} in Scheme 9, p. 23) and Stevens and Whiting<sup>78</sup> (all-*trans-}36\text{c} and **-44** in Scheme 11, p. 24) were quite similar to our product. A NOESY experiment of the product showed***

correlation between H-7'/8, 7'/9', 8/9' and 7/8', but there were no correlations between H-7'/8' or 8/8', which was good evidence for an all-*trans* structure. On the basis of these results it was concluded that the rearranged product was a 7',8'-*trans*-8,8'-*trans* (7'*S*,8*S*,8'*R*) isomer all-*trans*-**36c**, which arised from a translactonisation and  $\alpha$ -epimerisation.<sup>1</sup> The unlabelled racemic form had been reported by Stevens and Whiting.<sup>78</sup>

### 6.3.2 Translactonisation studies with KOH/MeOH and NaH/DMF

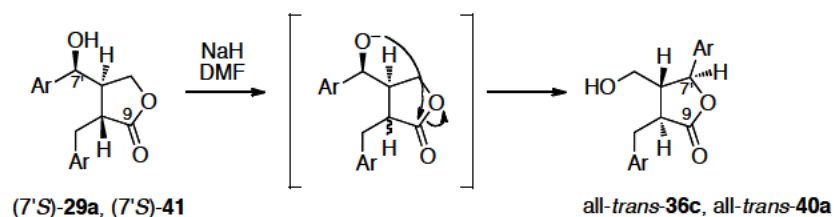
Due to the similarity in the outcome (a rearranged all-*trans* lactone) using different reaction conditions, the procedures of Niwa *et al.*<sup>65</sup> (2 % KOH in MeOH at rt; discussed above) and Moritani *et al.*<sup>71</sup> (NaH in DMF at 0°C; Scheme 9, p. 23) were compared. (–)-Parabenzlactone (7'*S*)-**29a** was submitted to both reaction conditions (Scheme 36). The KOH/MeOH furnished a mixture of starting material (7'*S*)-**29a** and rearranged lactone all-*trans*-**36c**, as expected. Surprisingly, a mixture of starting material and rearranged lactone was furnished also by NaH/DMF. Therefore, in our hands the reaction proved to behave very differently from Moritani's reaction (Scheme 9, p. 23), which showed a 75 % yield and no recovery or detection of starting material was reported.<sup>71</sup> To exclude any effect of the dissimilar aromatic substituents, both above mentioned rearrangement conditions were used also to Moritani's substrate (7'*S*)-**41** (Scheme 36) (with the exception of us having the enantiopure compound, while Moritani *et al.* used the racemic mixture).



**Scheme 36** Both reaction conditions yielded the same rearranged all-*trans*-lactone. For **29a** and **36c** R=R=OCH<sub>2</sub>O; for **40a** and **41** R=OMe.

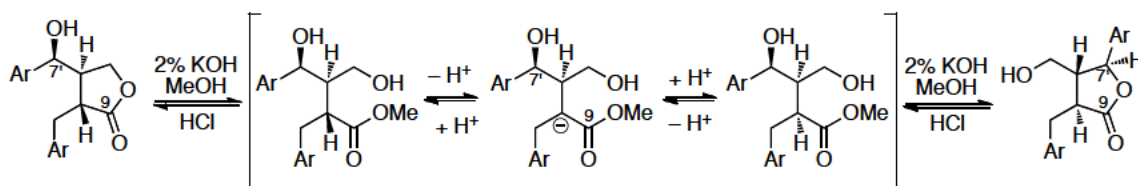
Both reactions led to a mixture of the starting material and the rearranged all-*trans*-lactone (**36c** from **29a** and **40a** from **41**) with doublet at  $\delta$  5.13 ppm,  $J$  = 9 Hz (H-7'). With NaH/DMF the ratio was about 40:60, and with KOH/MeOH about 60:40, irrespective of the aromatic substituents.<sup>1</sup> The difference in the predominant product obtained under different reaction conditions may be explained by the reaction mechanisms.

In the case of NaH/DMF (Scheme 37), the lactone moiety undergoes a nucleophilic attack by the formed alkoxide ion and the more or less concurrent  $\alpha$ -epimerisation gives the thermodynamically more stable all-*trans* product.\*\*



**Scheme 37** Translactonisation and  $\alpha$ -epimerisation in NaH/DMF.

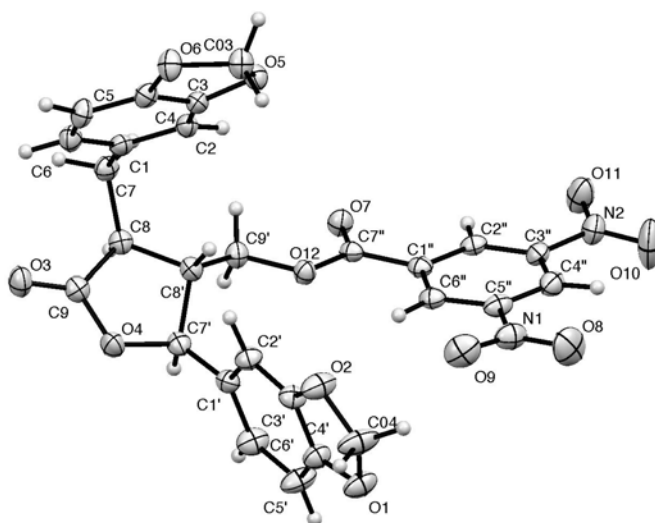
When treating lactone (7'S)-**29a** or (7'S)-**41** with KOH/MeOH, the lactone moiety undergoes transesterification by the action of  $\text{MeO}^-$  present in the reaction medium (Scheme 38). The methyl ester diol intermediate can be deprotonated at the  $\alpha$  position. On acidification, relactonisation can occur with either of the hydroxy groups in the intermediate. A reprotonation from the upper face leads back to the starting material, whereas protonation from the lower face will lead to the rearranged lactone. The ratio of starting material 9,9'-lactone and rearranged product 9,7'-lactone (60:40) indicates that the structure of 9,9'-lactone ((7'S)-**29a** or (7'S)-**41**) is preferred. A similar ratio was observed when the rearranged 9,7'-lactone all-*trans*-**36c** was treated with 2 % KOH/MeOH, but the equilibrium settled more slowly (48 h) than when (7'S)-**29a** was used as the starting material (1.5 h). Probably the rearranged 9,7'-lactone is more hindered than the 9,9'-lactone making transesterification less favorable. When (7'S)-**29a** and all-*trans*-**36c** were treated with 2 % KOH/*t*-BuOH no rearrangement was observed.



**Scheme 38** Translactonisation and  $\alpha$ -epimerisation in 2 % KOH/MeOH.<sup>†</sup>

The absolute configuration of all-*trans*-**36c** was finally confirmed also by X-ray analysis of its 3,5-dinitrobenzoate derivative **86** (Figure 19).

\*\* Sterical energies calculated using Merck Molecular Force Field were 25.447 kcal/mol, 26.598 kcal/mol and 29.598 kcal/mol for **29a**, all-*trans*-**36a** and 7',8'-*trans*-8,8'-*cis*-**36d**, respectively (unpublished results). Therefore the  $\alpha$ -epimerised product all-*trans*-**36a** is thermodynamically more favorable than the 7',8'-*trans*-8,8'-*cis*-**36d**, which would result from a mere translactonisation.

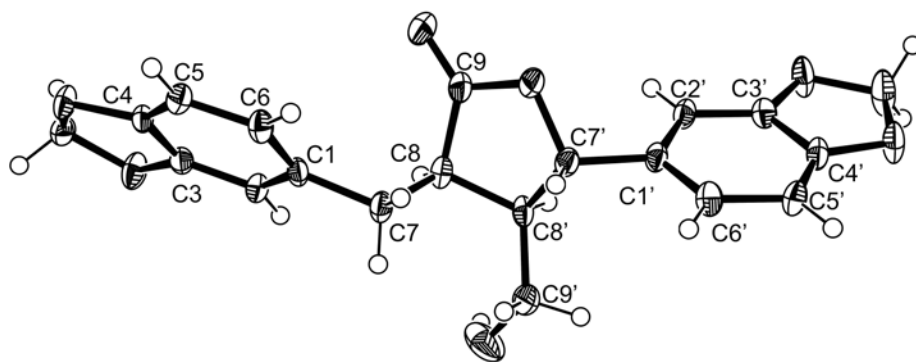


**Figure 19** X-ray crystal structure of all-*trans*-**86** (50 % probability ellipsoids). ORTEP plot.<sup>I</sup>

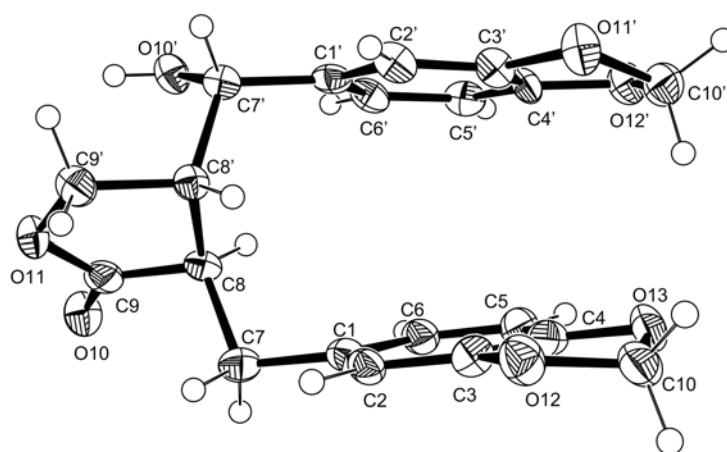
### 6.3.3 Crystal structures of (–)-parabenzlactone and rearranged regioisomer.

As mentioned in the Chapter 2.4, the reduction of 7'-oxolignano-9,9'-lactones with L-Selectride produced in most cases a byproduct identified as the rearranged lactone, in addition to the desired target (7'*S*)-OH-lignano-9,9'-lactone (Scheme 4, p. 20). Usually the rearranged lactone was only detected by NMR, but in the case of 7',8'-*trans*-8,8'-*cis*-**33a**,<sup>70</sup> 7',8'-*trans*-8,8'-*cis*-**36d** and 7',8'-*trans*-8,8'-*cis*-**40b** the products were isolated and characterised.<sup>I</sup> The 7',8'-*trans*-8,8'-*cis* stereochemistry was assigned by 2D NMR (COSY and NOESY) and on the basis of the presumed mechanism of formation: the 7'-alkoxyborane, formed in the L-Selectride reaction, attacks the lactone carbonyl producing a primary alcohol and translactonisation (Scheme 6, p. 22).

As discussed before, in the literature there was only one lactone homochiral to ours, but with different aromatic substitution pattern, isohydroxymatairesinol 7',8'-*trans*-8,8'-*cis*-**35a** reported by Eklund *et al.* (Scheme 5, p. 21).<sup>76</sup> This lactone, obtained from a basic treatment of 7'-hydroxymatairesinol **31** is formed by a different mechanism than 7',8'-*trans*-8,8'-*cis*-**33a**, -**36d** and -**40b**, but they all have a clear correlation between the spectroscopic data, as can be seen in the Table A2 in Appendix (p. 97). Compound 7',8'-*trans*-8,8'-*cis*-**33a** was converted to 7',8'-*trans*-8,8'-*cis*-**35a** and the spectroscopic data were in agreement with those reported.<sup>76</sup> Additionally, we were able to crystallise 7',8'-*trans*-8,8'-*cis*-**36d**, and the absolute configuration of the rearranged product was confirmed also with X-ray analysis (Figure 20), as well as the (7'*S*)-hydroxylignano-9,9'-lactone (–)-parabenzlactone (7'*S*)-**29a** (Figure 21).<sup>II</sup>



**Figure 20** Rearranged lactone 7',8'-*trans*-8,8'-*cis*-**36d** from L-Selectride reduction of **85**. ORTEP plot (50 % probability ellipsoids).<sup>II</sup>



**Figure 21** X-ray crystal structure of (-)-parabenzlactone (7'*S*)-**29a**. ORTEP plot (50 % probability ellipsoids).<sup>II</sup>

#### 6.3.4 Discussion on the all-*trans* and all-*cis* isomers of rearranged hydroxymatairesinol

With the correct stereochemistry of all-*trans*-**36c**,<sup>65,I</sup> the <sup>1</sup>H NMR spectra of all-*trans*- (entries 1–5, Table 2), 7',8'-*trans*-8,8'-*cis*- (entries 6–9) and 7',8'-*cis*-8,8'-*trans*-lignano-9,7'-lactones (entries 10–14) show a close resemblance, particularly regarding the diagnostic peak of H-7'. It is interesting to note that regardless of the alcohol being free or protected (TBDMS or Piv), there are no remarkable differences in the NMR spectra of the homochiral lignans. However, a similar comparison cannot be made in regard of all-*cis*-lignano-9,7'-lactones at the moment. There are only two all-*cis*-lignano-9,7'-lactones (entries 15 and 16) reported to date presenting a significant difference for the H-7' signals in both chemical shifts and coupling constants:  $\delta$  5.12 ppm, d,  $J_{\text{H-7'},\text{H-8'}} = 9.3$  Hz for all-*cis*-**35b**,<sup>76</sup> and  $\delta$  5.43 ppm, d,  $J_{\text{H-7'},\text{H-8'}} = 5.4$  Hz for all-*cis*-**45**.<sup>78</sup>



As speculated in the article I, the notable divergence of these values could be caused by the bulky TBDMS group, which may significantly affect the conformation of all-*cis*-**45**, even if similar behavior is not observed for other configurations. However, it is surprising that the spectroscopic data of all-*cis*-**35b** and of the all-*trans* isomers show notable similarity (entries 16 and 1–5, Table 2). Especially the all-*trans*-**35c** is nearly identical with its all-*cis* stereoisomer all-*cis*-**35b** (Table 3). The configuration of the piperonyl substituted analog of all-*trans*-**35c** is verified by X-ray analysis as all-*trans* (the 3,5-dinitrobenzoate derivative **86** of all-*trans*-**36c** in Figure 19).

The striking similarity tempts one to speculate whether the resemblance of spectroscopic data between all-*trans*-**35c** and all-*cis*-**35b** is just a coincidence, or if the reported all-*cis*-**35b** might be all-*trans* instead. Eklund *et al.* have presented convincing evidence (NOESY study and mechanism of formation) that the minor product is the all-*cis*-lignano-9,7'-lactone.<sup>76</sup> However, the conclusion by deducing the configuration from the coupling constant between H-7' and H-8' (9.3 Hz for *cis* and 2.6 Hz for *trans*) is somewhat ambiguous, since the coupling constants of both the magnitudes (8.5–9.5 Hz and 2.0–2.6 Hz) have been observed for 7',8'-*trans* configuration (Table 2, entries 1–5 for all-*trans*-structures, and entries 6–9 for 7',8'-*trans*-8,8'-*cis*-hydroxylignano-9,7'-lactones).

**Table 2.** Synthetic 9'-hydroxylignano-9,7'-lactones.<sup>††</sup>

entry	structure	stereochemistry	data	ref.
			H NMR, 7'H ( $\delta$ in CDCl <sub>3</sub> )	
all- <i>trans</i>				
1		7'S*,8S*,8'R* all- <i>trans</i> - <b>40a</b>	5.16 ppm d, <i>J</i> = 9.2 Hz	71,I
2		7'S*,8S*,8'R* all- <i>trans</i> - <b>44</b>	5.13 ppm d, <i>J</i> = 9.2 Hz	78
3		7'S*,8S*,8'R* all- <i>trans</i> - <b>36c</b>	5.14 ppm d, <i>J</i> = 9.0 Hz	78
		7'S,8S,8'R all- <i>trans</i> - <b>36c</b>	5.13 ppm d, <i>J</i> = 8.5 Hz	I,65
4		7'S,8S,8'R all- <i>trans</i> - <b>35c</b>	5.11 ppm d, <i>J</i> = 9.5 Hz	I
5		7'S,8S,8'R all- <i>trans</i> - <b>87</b>	5.11 ppm d, <i>J</i> = 9.0 Hz	I

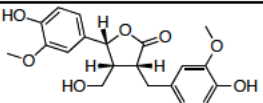
<sup>††</sup> In Appendix, pp. 94–96, there is an extended version of Table 2: Table A1 with mechanisms of formation and optical rotation data of the reported synthetic 9'-hydroxylignano-9,7'-lactones.



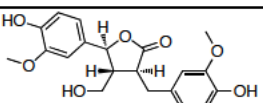
Table 2 continues

<i>7',8'-trans-8,8'-cis</i>				
6		<i>7'S,8R,8'R</i> <i>7',8'-trans-8,8'-cis-35a</i>	5.46 ppm d, <i>J</i> = 2.6 Hz	76
7		<i>7'S,8R,8'R</i> <i>7',8'-trans-8,8'-cis-33a</i>	5.45 ppm d, <i>J</i> = 2.6 Hz	70
8		<i>7'S,8R,8'R</i> <i>7',8'-trans-8,8'-cis-40b</i>	5.53 ppm d, <i>J</i> = 2.0 Hz	I
9		<i>7'S,8R,8'R</i> <i>7',8'-trans-8,8'-cis-36d</i>	5.49 ppm d, <i>J</i> = 2.0 Hz	II
<i>7',8'-cis-8,8'-trans</i>				
10		<i>7'R,8S,8'R</i> <i>7',8'-cis-8,8'-trans-43</i>	5.38 ppm d, <i>J</i> = 7.3 Hz	77
11		<i>7'S,8R,8'S</i> <i>7',8'-cis-8,8'-trans-88</i>	5.32 ppm d, <i>J</i> = 6.8 Hz	54
12		<i>7'R,8S,8'R</i> <i>7',8'-cis-8,8'-trans-36b</i>	5.38 ppm d, <i>J</i> = 7.8 Hz	77
13		<i>7'S,8R,8'S</i> <i>7',8'-cis-8,8'-trans-89</i>	5.32 ppm d, <i>J</i> = 7.7 Hz	60
14		<i>7'S,8R,8'S</i> <i>7',8'-cis-8,8'-trans-90</i>	5.35 ppm d, <i>J</i> = 7.8 Hz	202
<i>all-cis</i>				
15		<i>7'R*,8R*,8'R*</i> <i>all-cis-45</i>	5.43 ppm d, <i>J</i> = 5.4 Hz	78
16		<i>7'R,8R,8'R</i> <i>all-cis-35b</i>	5.12 ppm d, <i>J</i> = 9.3 Hz	76

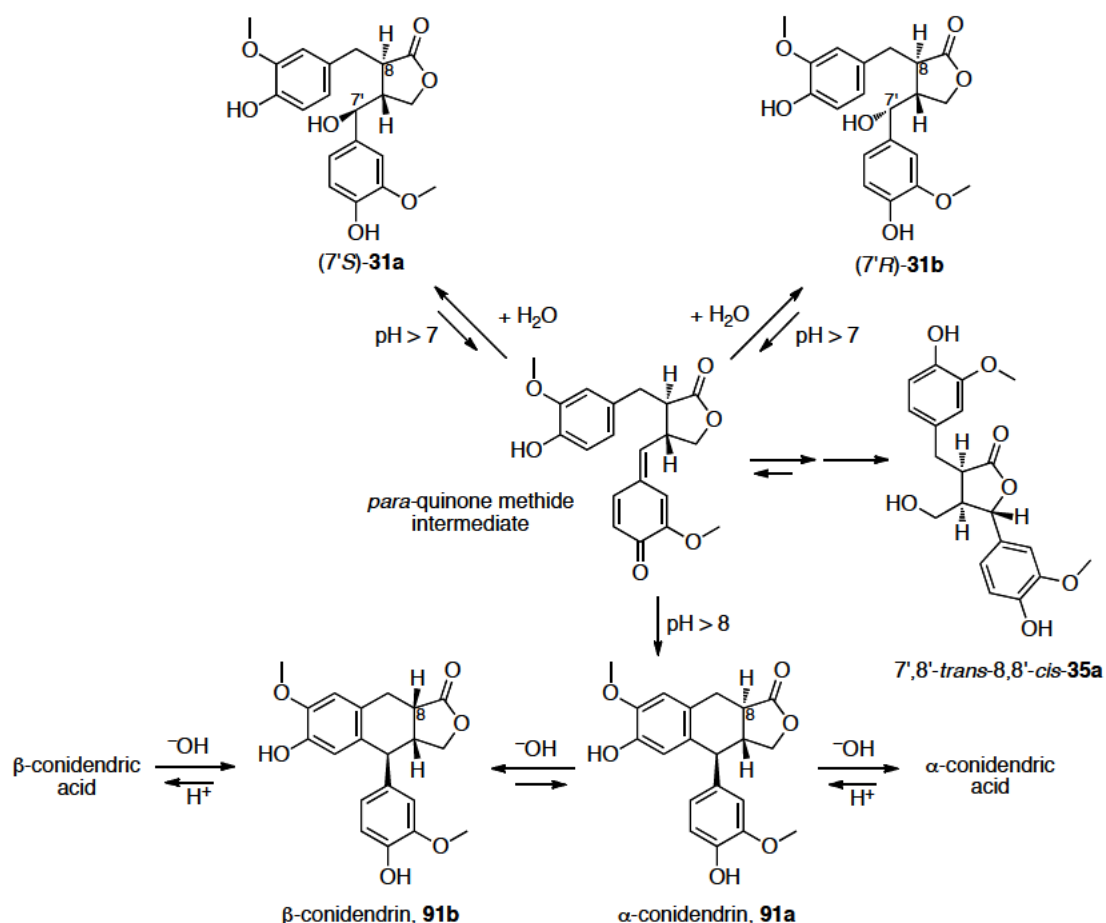
**Table 3.** The  $^1\text{H}$ -NMR and optical rotation data of *all-cis-35b* and *all-trans-35c*.

 <div> <b><i>all-cis-35b</i><sup>76</sup></b>  <math>[\alpha]_{\text{D}}^{25} +59.5</math> (<i>c</i> 0.005, THF)         </div>														
$^1\text{H}$ NMR (500 MHz, $\text{CDCl}_3$ )														
$\delta$	6.83	6.82	6.81	6.70	6.69	6.49	5.12	3.86	3.76	3.57	3.50	3.12	3.08	2.30
ppm														
m	d	d	d	ddd	dd	d	d	s	s	ddd	ddd	ddd	d	dddd
<i>J</i> (Hz)	8.1	8.0	2	2 8.1 0.4	2.0 8.0	2.0	9.3			3.9 4.0 11.0	3.9 4.0 11.0	5.8 5.8 10.2	5.8	4.0 4.1 9.3 10.2
H	1 H-5'	1 H-5	1 H-2	1 H-6'	1 H-6	1 H-2'	1 H-7'	3 OCH <sub>3</sub>	3 OCH <sub>3</sub> '	1 H-9'	1 H-9'	1 H-8	2 H-7	1 H-8'

 <div> <b><i>all-trans-35c</i><sup>1</sup></b>  <math>[\alpha]_{\text{D}}^{27} +52.68</math> (<i>c</i> 0.2, THF)         </div>														
$^1\text{H}$ NMR (500 MHz, $\text{CDCl}_3$ )														
$\delta$	6.84	6.83	6.81	6.70	6.69	6.49	5.11	3.87	3.77	3.58	3.50	3.14–3.05		2.32–2.27
ppm														
m	d	d	d	ddd	dd	d	d	s	s	dd	dd	m		m
<i>J</i> (Hz)	8.1	8.0	1.5	2.0 8.0	1.5 8.0	2.0	9.5			3.5 11.0	4.0 11.0			
H	1	1	1	1	1	1	1 H-7'	3	3	1	1	3		1

In a paper on the formation and reactivity of the quinone methide intermediate in the reactions of hydroxymatairesinol it was reported that when pure samples of hydroxymatairesinol isomers (7'*S*)-**31a** and (7'*R*)-**31b** are treated by an alkaline aqueous solution, a mixture of  $\alpha$ -conidendric acid,  $\beta$ -conidendric acid,  $\alpha$ -conidendrin **91a**, 7',8'-*trans*-8,8'-*cis*-**35a** and other products is formed (Scheme 39).<sup>203</sup> The formation of  $\beta$ -conidendrin **91b** occurs via epimerisation at C-8,  $\alpha$  to the lactone carbonyl. In the case of  $\alpha$ -conidendrin **91a**, the formation of the six-membered aliphatic ring probably increases the strain in the lactone ring, and the  $\alpha$  epimerisation allows less tension in the ring structure.<sup>204</sup> The case of the rearranged 9'-hydroxylignano-9,7'-lactone is different and most likely less strained, however; the  $\alpha$  epimerisation of 7',8'-*trans*-8,8'-*cis*-**35a** would lead to an *all-trans* structure, namely *all-trans*-**35c**. Thus one possible explanation is that in the *p*-quinone methide mechanism (Scheme 5, p. 21) the translactonisation affords only one rearranged product, i.e. 7',8'-*trans*-8,8'-*cis*-**35a**, and part of it epimerises to *all-trans*-**35c**.



**Scheme 39** Part of the observed transformations of 7'-hydroxymatairesinol **31** in alkaline aqueous solution. The composition of the mixture (major products and ratio) vary depending on the pH of the solution. Reaction at pH 10 yielded α- and β-conidendric acids as the main products.<sup>203</sup> The formation of β-conidendrin **91b** takes place via epimerisation at C-8.

Additionally, a tentative identification of the 8-epimer of isohydroxymatairesinol (7',8'-*trans*-8,8'-*cis*-**35a**) in the extractives of spruce knotwood was reported.<sup>76</sup> The 8-epimer would actually be the all-*trans*-**35c**, with NMR-data identical to those of the reported all-*cis*-**35b**. Thus, these two minor compounds, all-*cis*-**35b** and all-*trans*-**35c**, the 8-epimer of 7',8'-*trans*-8,8'-*cis*-**35a**, would be indistinguishable in NMR. However, this is all speculation since there are only two examples of all-*cis* stereochemistry, and the resemblance of the spectroscopic data between all-*trans*-**35c** and all-*cis*-**35b** can be an interesting coincidence. The X-ray analysis of an all-*cis* lactone would be the answer to the presented problem.

## 7 Conclusions

Deuteration of various lignano-9,9'-lactones and 9,9'-epoxylignanes was studied, and eleven new stable deuterium labelled lignans with good yields and high isotopic purity were synthesised. The method using  $D_3PO_4 \cdot BF_3 / D_2O$  as deuteration reagent was used to examine the relative ease of H/D exchange at various aromatic positions, and the observed results were compared with calculated reactivities. When deutrating with DCl in  $D_2O$ , the lignan dehydroxycubebin **84** gave unexpected results, and computational studies were performed to understand the deviating behavior from other close lignan analogues.

The development of effective and fast deuteration methods is highly important since the availability of labelled compounds is usually the limiting factor in the development and use of quantitative methods. The results in deutrating lignans, in terms of isotopic purity, are rather good regardless of the acidic deuteration reagent used. However, the reaction times are often quite long, probably resulting from solubility issues, but the method using DCl in  $D_2O$  and an ionic liquid as co-solvent reduced the reaction times significantly.

In our laboratory, while developing and optimising a stereoselective protocol to synthesise (7'*S*)-hydroxylignano-9,9'-lactones, an unexpected rearranged 7',8'-*trans*-8,8'-*cis*-9,7'-lactone was obtained as a byproduct, formed via translactonisation mechanism. The absolute configuration of the rearranged products as well as the 7'-hydroxylignano-9,9'-lactones were confirmed by X-ray crystallography of **36d** and (–)-parabenzlactone (7'*S*)-**29a**. Similar 9,7'-lactones, but with different stereochemistry and different formation mechanism, were reported in the literature. Thus the rearrangement reactions of various hydroxylignano-9,9'-lactones and 9,7'-lactones were investigated. Mechanistic studies with  $^{18}O$  labelled substrates supported the proposed translactonisation and *p*-quinone methide mechanisms in the formation of 7',8'-*trans*-8,8'-*cis*-lignano-9,7'-lactones, but showed that both the presumed mechanism and the stereochemistry of the rearranged lactone obtained by treating (7'*S*)-acetylparabenzlactone with 2 % KOH/MeOH were erroneous. The rearranged compound **36c** with an all-*trans*-stereochemistry was derived from a translactonisation with  $\alpha$ -epimerisation, driven by a relief of steric constraints in the rearranging lactone. The absolute configuration of the rearranged product **36c** was confirmed by X-ray analysis of its dinitrobenzoate derivative all-*trans*-**86**. The crystal structures for (7'*S*,8*R*,8'*R*)-7'-hydroxylignano-9,9'-lactones, and 9'-hydroxylignano-9,7'-lactones possessing stereochemistry (7'*S*,8*R*,8'*R*) and (7'*S*,8*S*,8'*R*) are the first reported for these kind of structures, probably due to the amorphous-like texture of lignans, which makes them demanding to crystallise.

## 8 Experimental

The general procedure for the synthesis of 9,9'-epoxylignanes from corresponding lignane-9,9'-diols was described in article V. Here the spectral data of the synthesised 9,9'-epoxylignanes is reported.

The described conversion from lignane-9,9'-diol to a tetrahydrofuran lignan with conc. HCl under microwave heating is in most cases completed in less than 30 minutes, which makes it a fast and easy cyclisation method, compared with conventional heating methods. In most of the reviewed<sup>V</sup> conventional methods (e.g. refluxing with methanolic HCl, or stirring with *p*-toluenesulfonyl chloride in pyridine) the cyclisation of dibenzyl-1,4-butanediols to 3,4-dibenzyl-tetrahydrofurans takes several hours.

Additionally, we have anticipated that our cyclisation method can work well also for synthesising the furofuran structures from tetraols.

### 9,9'-epoxylignane (8*R*\*,8'*R*'-6)

<sup>1</sup>H-NMR (300 MHz, CDCl<sub>3</sub>) δ 2.17–2.29 (2H, m, H-8, 8'), 2.57 (2H, dd, *J* = 8.4, 13.8, H-7a, 7'a), 2.70 (2H, dd, *J* = 5.7, 13.8, H-7b, 7'b), 3.52 (2H, dd, *J* = 6.6, 8.7, H-9a, 9'a), 3.89 (2H, dd, *J* = 6.6, 8.7, H-9b, 9'b), 7.08–7.29 (10H, m, Ar-H).

<sup>13</sup>C-NMR (75 MHz, CDCl<sub>3</sub>) δ 39.6 (C-7, 7'), 46.8 (C-8, 8'), 73.6 (C-9, 9'), 126.4 (C-4, 4'), 128.7 (C-2, 2', 6, 6'), 128.9 (C-3, 3', 5, 5'), 140.6 (C-1, 1').

EIMS (70 eV) *m/z*: M<sup>+</sup> 252 (15 %), 234 (15), 161 (3), 143 (18), 92 (100), 91 (65). HRMS (EI): *m/z* calc. for C<sub>18</sub>H<sub>20</sub>O (M<sup>+</sup>) 252.1514, found 252.1505.

### Anhydrosecoisolariciresinol (8*R*\*,8'*R*'-24)

#### 4,4'-dihydroxy-3,3'-dimethoxy-9,9'-epoxylignane

<sup>1</sup>H-NMR (300 MHz, *d*<sub>6</sub>-acetone) δ 2.12–2.24 (2H, m, H-8, 8'), 2.49 (2H, dd, *J* = 8.4, 13.5, H-7a, 7'a), 2.60 (2H, dd, *J* = 6.3, 13.5, H-7b, 7'b), 3.43 (2H, dd, *J* = 6.3, 8.7, H-9a, 9'a), 3.81 (6H, s, 3-, 3'-OMe), 3.81 (2H, dd, *J* = 6.6, 15.3, H-9b, 9'b), 6.61 (2H, dd, *J* = 1.8, 8.1, H-6, 6'), 6.73 (2H, d, *J* = 8.1, H-5, 5'), 6.73 (2H, d, *J* = 1.8, H-2, 2').

<sup>13</sup>C-NMR (75 MHz, *d*<sub>6</sub>-acetone) δ 39.6 (C-7, 7'), 47.7 (C-8, 8'), 56.2 (3-, 3'-OMe), 73.8 (C-9, 9'), 113.1 (C-2, 2'), 115.6 (C-5, 5'), 122.0 (C-6, 6'), 133.1 (C-1, 1'), 145.7 (C-4, 4'), 148.2 (C-3, 3').

EIMS (70 eV) *m/z*: M<sup>+</sup> 344 (59 %), 314 (8), 207 (6), 138 (77), 137 (100), 123 (12), 122 (13), 106 (14). HRMS (EI): *m/z* calc. for C<sub>20</sub>H<sub>24</sub>O<sub>5</sub> (M<sup>+</sup>) 344.4016, found 344.1629.

### Enterofuran (8*R*\*,8'*R*'-54)

#### 3,3'-dihydroxy-9,9'-epoxylignane

<sup>1</sup>H-NMR (500 MHz, *d*<sub>6</sub>-acetone) δ 2.16–2.23 (2H, m, H-8, 8'), 2.50 (2H, dd, *J* = 9.0, 13.5, H-7a, 7'a), 2.67 (2H, dd, *J* = 5.5, 13.5, H-7b, 7'b), 3.43 (2H, dd, *J* = 6.8,

8.5, H-9a, 9'a), 3.79 (2H, dd,  $J = 6.8$ , 8.5, H-9b, 9'b), 6.64–6.68 (6H, m, H-2, 4, 6, 2', 4', 6'), 7.09 (2H, t,  $J = 7.8$ , H-5, 5'), 8.15 (2H, s, 3-OH, 3'-OH).

$^{13}\text{C}$ -NMR (75 MHz,  $d_6$ -acetone)  $\delta$  39.8 (C-7, 7'), 47.5 (C-8, 8'), 73.7 (C-9, 9'), 113.8 (C-4, 4'), 116.5 (C-2, 2'), 120.7 (C-6, 6'), 130.2 (C-5, 5'), 143.3 (C-1, 1'), 158.3 (C-3, 3').

HRMS (EI):  $m/z$  calc. for  $\text{C}_{18}\text{H}_{20}\text{O}_3$  ( $\text{M}^+$ ) 284.1412, found 284.1424.

Brassilignan (8*R*\*,8'*R*'\*-**83**)

3,3',4,4'-tetramethoxy-9,9'-epoxylignan

$^1\text{H}$ -NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  2.14–2.26 (2H, m, H-8, 8'), 2.53 (2H, dd,  $J = 8.1$ , 13.8, H-7a, 7'a), 2.64 (2H, dd,  $J = 6.0$ , 13.8, H-7b, 7'b), 3.53 (2H, dd,  $J = 6.0$ , 9.0, H-9a, 9'a), 3.84 (6H, s,  $\text{OCH}_3$ ), 3.85 (6H, s,  $\text{OCH}_3$ ), 3.91 (2H, dd,  $J = 6.6$ , 8.7, H-9b, 9'b), 6.59 (2H, d,  $J = 1.8$ , H-2, 2'), 6.63 (2H, dd,  $J = 1.8$ , 8.1, H-6, 6'), 6.76 (2H, d,  $J = 8.1$ , H-5, 5').

$^{13}\text{C}$ -NMR (125 MHz,  $\text{CDCl}_3$ )  $\delta$  39.1 (C-7, 7'), 46.6 (C-8, 8'), 55.8 ( $\text{OCH}_3$ ), 55.9 ( $\text{OCH}_3$ ), 73.3 (C-9, 9'), 111.2 (C-5, 5'), 112.0 (C-2, 2'), 120.6 (C-6, 6'), 133.0 (C-1, 1'), 147.4 (C-4, 4'), 148.9 (C-3, 3').

EIMS (70 eV)  $m/z$ :  $\text{M}^+$  372 (75 %), 177 (5), 152 (87), 151 (100), 137 (11), 121 (13). HRMS (EI):  $m/z$  calc. for  $\text{C}_{22}\text{H}_{28}\text{O}_5$  ( $\text{M}^+$ ) 372.1937, found 372.1938.

Dehydroxycubebin (8*R*\*,8'*R*'\*-**84**)

(3,4),(3',4')-bis(methylenedioxy)-9,9'-epoxylignan

$^1\text{H}$ -NMR (300 MHz,  $d_6$ -acetone)  $\delta$  2.10–2.22 (2H, m, H-8, 8'), 2.50 (2H, dd,  $J = 7.8$ , 13.5, H-7a, 7'a), 2.60 (2H, dd,  $J = 6.6$ , 13.5, H-7b, 7'b), 3.42 (2H, dd,  $J = 6.0$ , 8.4, H-9a, 9'a), 3.80 (2H, dd,  $J = 6.9$ , 8.4, H-9b, 9'b), 5.94 (4H, dd,  $J = 1.1$  Hz, 2 x O-CH<sub>2</sub>-O), 6.61 (2H, dd,  $J = 1.5$ , 7.8, H-6, 6'), 6.65 (2H, d,  $J = 1.2$ , H-2, 2'), 6.72 (2H, d,  $J = 7.8$ , H-5, 5').

$^{13}\text{C}$ -NMR (125 MHz,  $d_6$ -acetone)  $\delta$  39.7 (C-7, 7'), 47.5 (C-8, 8'), 73.7 (C-9, 9'), 101.7 (C-10, 10'), 108.7 (C-5, 5'), 109.8 (C-2, 2'), 122.4 (C-6, 6'), 135.5 (C-1, 1'), 146.8 (C-4, 4'), 148.6 (C-3, 3').

EIMS (70 eV)  $m/z$ : 341 (18 %),  $\text{M}^+$  340 (69), 204 (4), 161 (16), 136 (100), 135 (93). HRMS (EI):  $m/z$  calc. for  $\text{C}_{20}\text{H}_{20}\text{O}_5$  ( $\text{M}^+$ ) 340.1311, found 340.1316.



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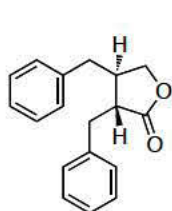
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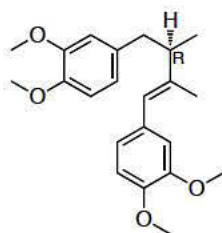
# Appendix

## Appendix 1. Lignan structures

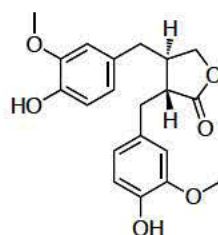
Certain skeletal formulas are assigned identification numbers. The presence of different stereoisomers and labelled isomers are expressed with prefixes.



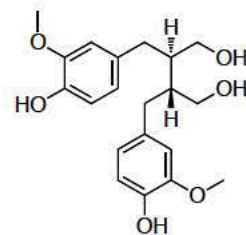
**5**



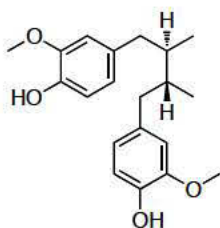
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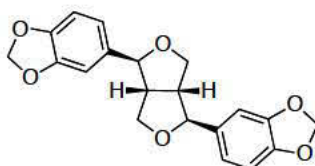
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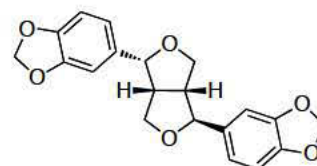
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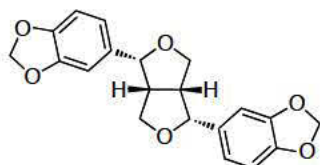
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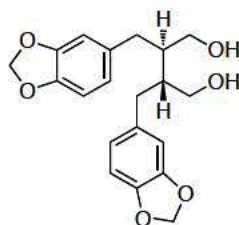
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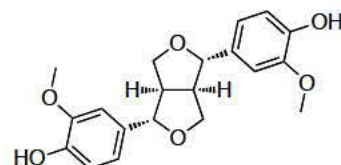
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(+)-(7*S*,7'*R*,8*R*,8'*R*)-**20b**



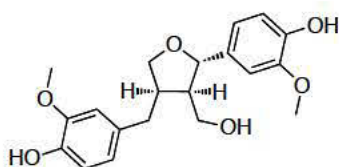
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(+)-(7*R*,7'*R*,8*R*,8'*R*)-**20c**



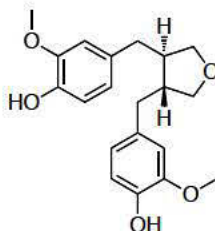
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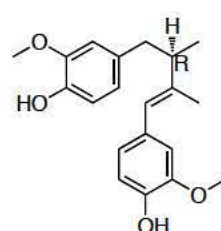
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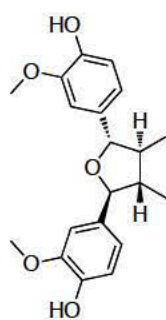
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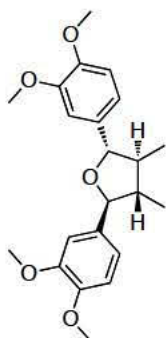
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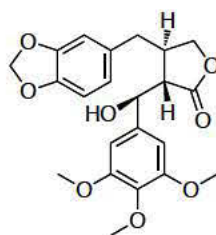
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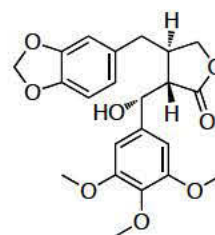
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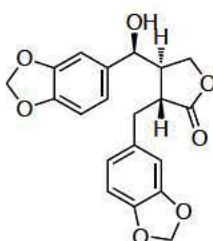
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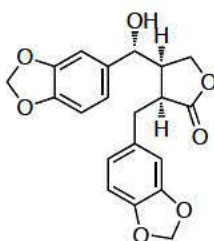
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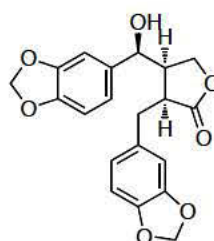
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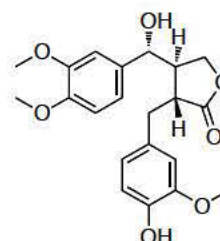
(-)-parabenzlactone,  
(7'*S*,8*R*,8'*R*)-**29a**



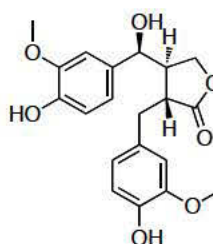
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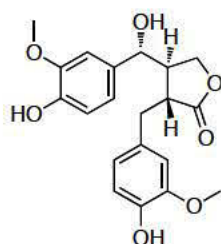
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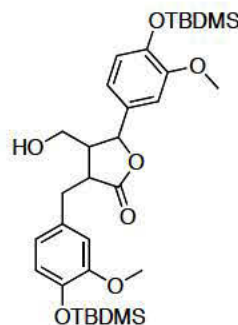
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(+)-(7'*R*)-**30**



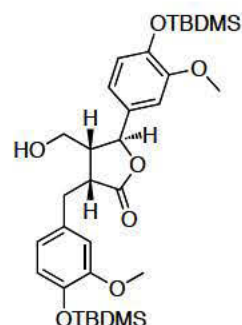
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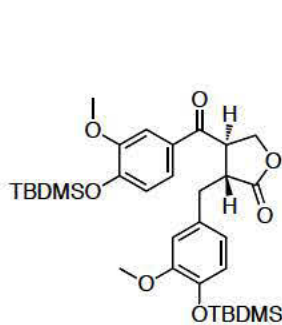
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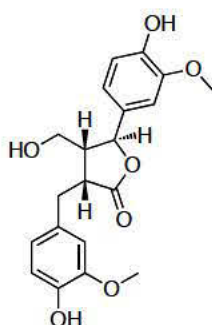
**33**



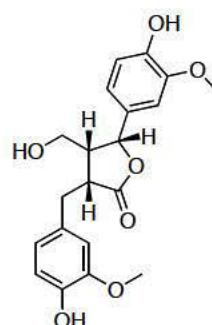
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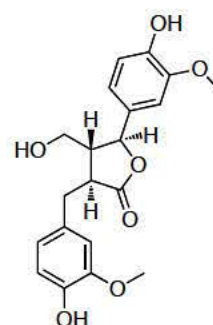
**32**



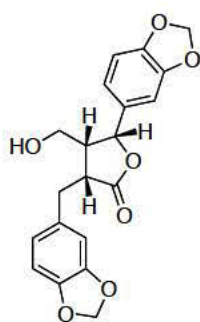
isohydroxymatairesinol,  
7',8'-*trans*-8,8'-*cis*-**35a**



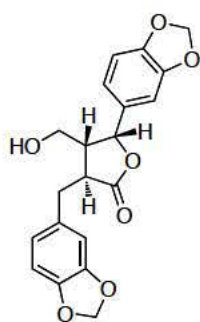
epi-isohydroxymatairesinol  
all-*cis*-**35b**



all-*trans*-**35c**



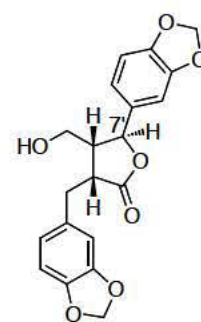
**all-cis-36a**



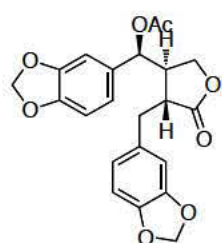
**7',8'-cis-8,8'-trans-36b**



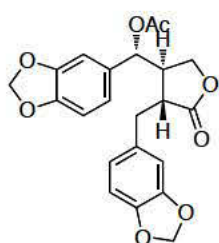
**all-trans-36c**



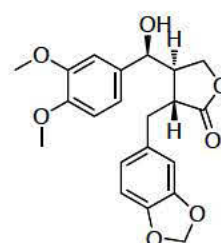
**7',8'-trans-8,8'-cis-36d**



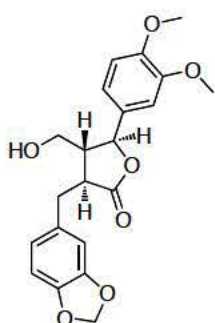
**acetylparabenzlactone, (7'S)-37a**



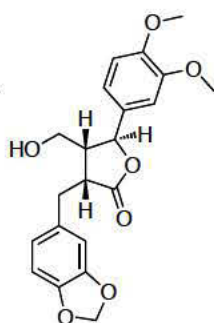
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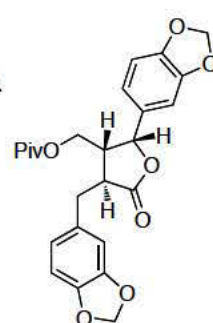
**(7'S)-41**



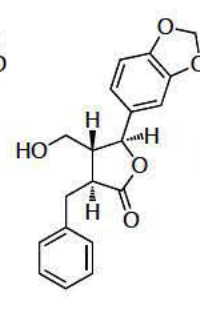
**all-trans-40a**



**7',8'-trans-8,8'-cis-40b**



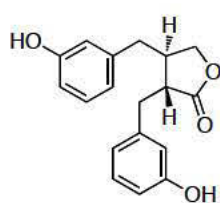
**7',8'-cis-8,8'-trans-43**



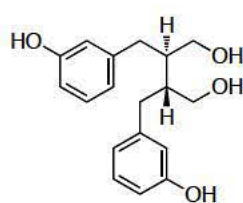
**all-trans-44**



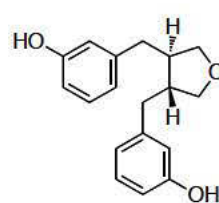
**all-cis-45**



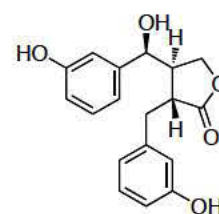
**enterolactone, 52**



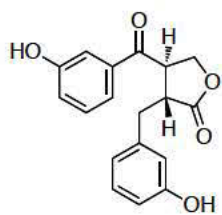
**enterodiols, 53**



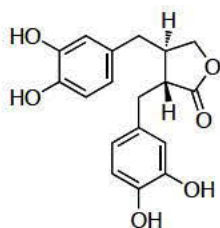
**enterofuran, 54**



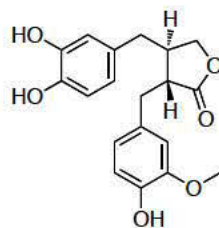
**7'-hydroxyenterolactone, 55 (7'S)-55a**



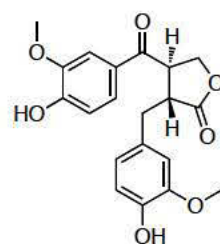
7'-oxoenterolactone, **56**



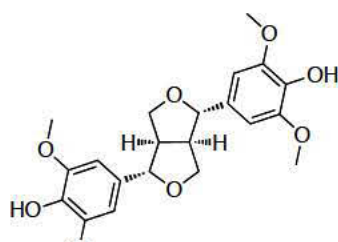
4,4'-dihydroxyenterolactone, **57**



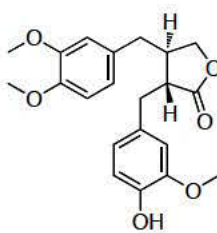
monodemethylated  
matairesinol, **58**



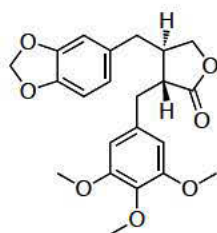
7'-oxomatairesinol, **59**



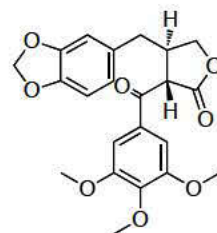
syringaresinol, (+)-**60**



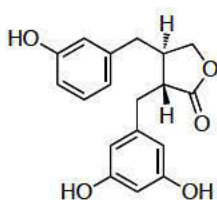
arctigenin, (-)-**61**



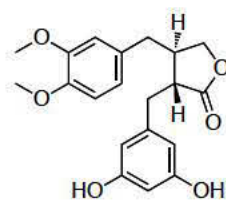
yatein, **66**



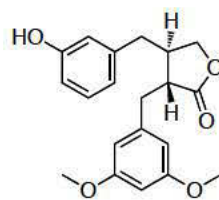
podorhizone, **67**



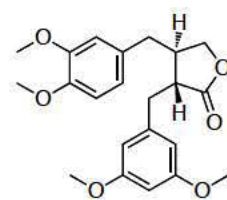
**76**



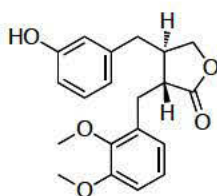
**77**



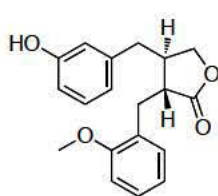
**78**



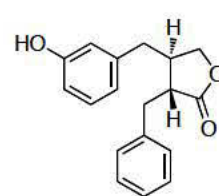
**79**



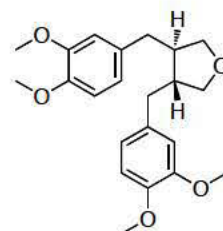
**80**



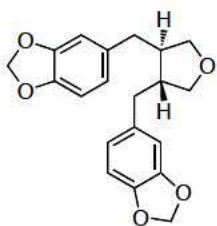
**81**



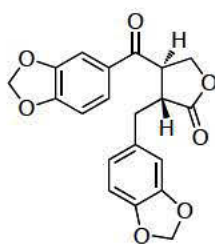
**82**



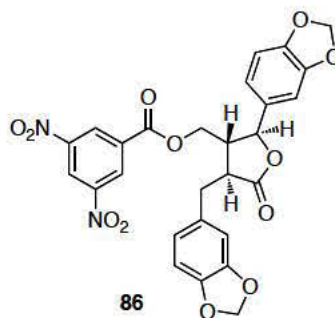
brassilignan, **83**



dehydroxycubebin, **84**

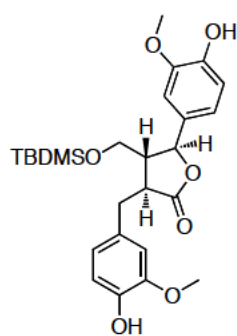


**85**

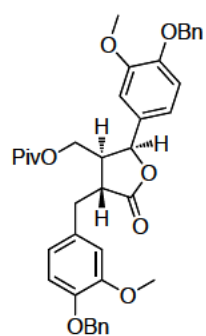


**86**

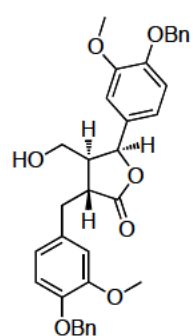




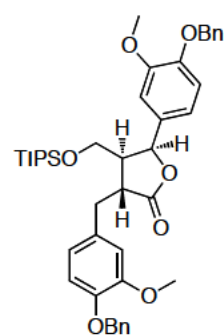
*all-trans*-**87**



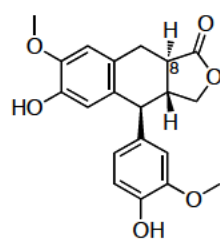
*7',8'-cis-8,8'-trans*-**88**



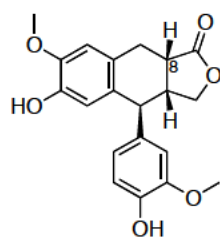
*7',8'-cis-8,8'-trans*-**89**



*7',8'-cis-8,8'-trans*-**90**



$\alpha$ -conidendrin, **91a**



$\beta$ -conidendrin, **91b**

## Appendix 2. 9'-Hydroxylignano-9,7'-lactones

Table A1. Synthetic 9'-hydroxylignano-9,7'-lactones.

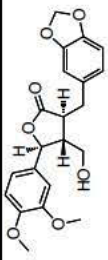
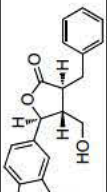
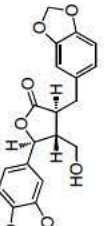
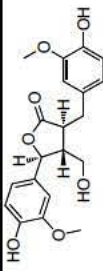
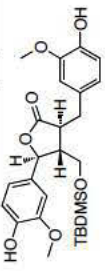
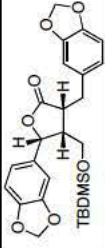
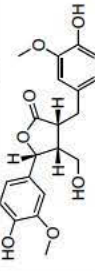
Entry	Structure	Stereochemistry	Mechanism of formation	Data		Ref.
				H NMR, 7'H ( $\delta$ in CDCl <sub>3</sub> )	[ $\alpha$ ] <sub>D</sub>	
all- <i>trans</i>						
1		7'S*,8S*,8'R* <sup>a</sup> all- <i>trans</i> - <b>40a</b>	rearrangement of a 7'( <i>S</i> )-OH-lignan lactone (NaH/DMF) via translactonisation and $\alpha$ -epimerisation at C-8	5.16 ppm d, <i>J</i> = 9.2 Hz	racemic	71
		7'S,8S,8' <i>R</i> all- <i>trans</i> - <b>40a</b>			+47.45° <i>c</i> = 1.08, THF	I
2		7'S*,8S*,8' <i>R</i> * all- <i>trans</i> - <b>44</b>	not a rearrangement product, but reduction of an arylidene lactone: stereochemistry confirmed by X-ray crystallography and subsequent chemistry	5.13 ppm d, <i>J</i> = 9.2 Hz	racemic	78
3		7'S*,8S*,8' <i>R</i> * all- <i>trans</i> - <b>36c</b>	not a rearrangement product, but reduction of an arylidene lactone: stereochemistry confirmed by X-ray crystallography and subsequent chemistry	5.14 ppm d, <i>J</i> = 9.0 Hz	racemic	78
		7'S,8S,8' <i>R</i> <sub>a,c</sub> all- <i>trans</i> - <b>36c</b>	rearrangement of 7' <i>S</i> -parabenzlactone (KOH/MeOH) via translactonisation and $\alpha$ -epimerisation			
4		7'S,8S,8' <i>R</i> all- <i>trans</i> - <b>35c</b>	rearrangement of a 7'( <i>S</i> )-OH-lignan lactone (NaH/DMF) via translactonisation and $\alpha$ -epimerisation	5.11 ppm d, <i>J</i> = 9.5 Hz	+52.68° <i>c</i> = 0.2, THF	I
5		7'S,8S,8' <i>R</i> all- <i>trans</i> - <b>87</b>	rearrangement of a 7'( <i>S</i> )-OH-lignan lactone (NaH/DMF) via translactonisation and $\alpha$ -epimerisation (migration of the phenolic protective silyl groups)	5.11 ppm d, <i>J</i> = 9.0 Hz	+36.83° <i>c</i> = 0.57, THF	I

Table A1 continues

7',8'-trans-8,8'-cis						
6		7'S,8R,8'R 7',8'-trans-8,8'-cis- <b>35a</b>	rearranged from 7'-hydroxymatairesinol (NaOH) in THF-H <sub>2</sub> O, pH 8) via <i>para</i> -quinone methide intermediate	5.46 ppm d, <i>J</i> = 2.6 Hz	+51.0° c=0.005, THF	76
7		7'S,8R,8'R 7',8'-trans-8,8'-cis- <b>33a</b>	rearranged during L-Selectride reduction of 7'-oxo-lignan lactone via transactonisation (no epimerisation)	5.45 ppm d, <i>J</i> = 2.6 Hz	+37.5° c=0.3, CHCl <sub>3</sub>	70
8		7'S,8R,8'R 7',8'-trans-8,8'-cis- <b>40b</b>	rearranged during L-Selectride reduction of (-)-parabenzlactone via transactonisation (no epimerisation)	5.53 ppm d, <i>J</i> = 2.0 Hz	+44.47° c=0.54, THF	I
9		7'S,8R,8'R <sup>a</sup> 7',8'-trans-8,8'-cis- <b>36d</b>	rearranged during L-Selectride reduction of (-)-parabenzlactone via transactonisation (no epimerisation)	5.49 ppm d, <i>J</i> = 2.0 Hz	+49.3° c=0.3, THF	II
7',8'-cis-8,8'-trans						
10		7'R,8S,8'R 7',8'-cis-8,8'-trans- <b>43</b>	not a rearrangement product, but an intermediate, prepared stereoselectively from L-arabinose, in the synthesis of <i>cis</i> -(7'R,8S,8'R)-parabenzlactone	5.38 ppm d, <i>J</i> = 7.3 Hz	-46.8° c=1.52, CHCl <sub>3</sub>	77
11		7'S,8R,8'S 7',8'-cis-8,8'-trans- <b>88</b>	not a rearrangement product, but an intermediate in the synthesis of an aryltetrahydronaphthalene type lignan	5.32 ppm d, <i>J</i> = 6.8 Hz	+30° c=0.6, CHCl <sub>3</sub>	54
12		7'R,8S,8'R 7',8'-cis-8,8'-trans- <b>36b</b>	sideproduct in the synthesis of <i>cis</i> -(7'R,8S,8'R)-parabenzlactone, converted (i. NaOH/EtOH; ii. aq. HCl) to the wanted product via transactonisation (no epimerisation)	5.38 ppm d, <i>J</i> = 7.8 Hz	-42.1° c=1.07, CHCl <sub>3</sub>	77
13		7'S,8R,8'S 7',8'-cis-8,8'-trans- <b>89</b>	intermediate in the synthesis of <i>cis</i> -(8R,8'S)-matairesinol, partly converted (i. NaOH/EtOH; ii. aq. HCl) to <i>cis</i> -(7'S,8R,8'S)-hydroxymatairesinol via transactonisation (no epimerisation)	5.32 ppm d, <i>J</i> = 7.7 Hz	+20° c=1.6, CHCl <sub>3</sub>	60
14		7'S,8R,8'S 7',8'-cis-8,8'-trans- <b>90</b>	not a rearrangement product, silyl protected from 7',8'-cis-8,8'-trans- <b>94</b> <sup>60</sup>	5.35 ppm d, <i>J</i> = 7.8 Hz	+33° c=2.0, CHCl <sub>3</sub>	202

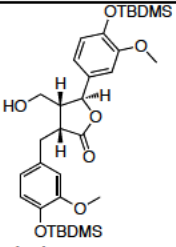
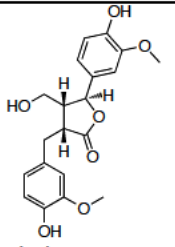
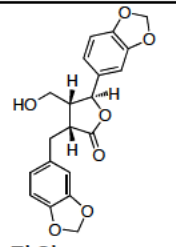
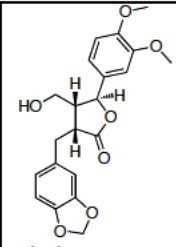
Table A1 continues

all-*cis*

15		7'R*,8R*,8'R* all- <i>cis</i> - <b>45</b>	not a rearrangement product but an intermediate in the synthesis of dihydrosesamin	5.43 ppm d, <i>J</i> = 5.4 Hz	racemic	78
16		7'R,8R,8'R all- <i>cis</i> - <b>35b</b>	rearranged from 7'-hydroxymatairesinol (NaOH in THF-H <sub>2</sub> O, pH 8) via <i>para</i> -quinone methide intermediate (no epimerisation)	5.12 ppm d, <i>J</i> = 9.3 Hz	+59.8° <i>c</i> =0.005, THF	76

<sup>a</sup> The structure is confirmed by X-ray crystallographic analysis.<sup>b</sup> Optical rotation for the oxygen-18 labelled analog was +66.97° (*c*=0.37, THF).<sup>1</sup><sup>c</sup> Niwa et al. assumed all-*cis*-stereochemistry,<sup>65</sup> but now corrected to all-*trans* and confirmed by X-ray analysis of the 3,5-dinitrobenzoate derivative.<sup>1</sup>

**Table A2.**  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ) data of 7',8'-trans-8,8'-cis-9'-hydroxylignano-9,7'-lactones.

Structure	 <b>7',8'-trans-8,8'-cis-33a</b>	 <b>7',8'-trans-8,8'-cis-35a</b>	 <b>7',8'-trans-8,8'-cis-36d</b>	 <b>7',8'-trans-8,8'-cis-40b</b>
$[\alpha]_D$	+37.5° ( $c=0.3$ , $\text{CHCl}_3$ )	+51.0° ( $c=0.005$ , THF)	+49.3° ( $c=0.3$ , THF)	+44.47° ( $c=0.54$ , THF)

"Body"	$\delta$ ; multipl.; $J$ (Hz)	$\delta$ ; multipl.; $J$ (Hz)	$\delta$ ; multipl.; $J$ (Hz)	$\delta$ ; multipl.; $J$ (Hz)
<b>H-7</b>	2.75; dd; 15.1, 10.6	2.76; dd; 15.0, 10.2	2.73; dd; 15.0, 10.5	2.74; dd; 15.0, 11.0
	3.23; dd; 15.1, 4.5	3.19; dd; 15.0, 5.0	3.22; dd; 15.0, 5.0	3.20; dd; 15.0, 5.0
<b>H-8</b>	3.09; ddd; 10.6, 8.2, 4.5	3.05; ddd; 10.2, 8.1, 5.0	3.04; ddd; 10.5, 8.0, 4.5	3.04; ddd; 11.0, 8.5, 5.0
<b>H-7'</b>	5.45; d; 2.6	5.46; d; 2.6	5.49; d; 2.0	5.53; d; 2.0
<b>H-8'</b>	2.59–2.63; m	2.60; dddd; 8.1, 6.9, 4.4, 2.6	2.56; ddd; 7.0, 4.5, 2.0	2.58–2.62; m
<b>H-9'</b>	3.73–3.78; m	3.76; dd; 10.7, 6.9	3.77; ddd; 10.5, 7.0, 5.5	3.78; dd; 11.0, 7.5
	3.93; dd; 10.5, 4.0	3.94; dd; 10.7, 4.4	3.95; dt; 10.5, 4.0	3.96; dd; 11.0, 4.5

<b>Aromatic protons</b>	6.65; dd; 8.0, 2.0 (1H)	6.67; dd; 8.1, 2.1	6.66; dd; 8.0, 1.5	6.65; dd; 8.0, 1.5
	6.71–6.73; m (2H)	6.72; d; 2.1	6.69; d; 1.5	6.69; d; 1.5
		6.75; ddd; 8.1, 2.0, 0.8	6.72; d; 8.0	6.71; d; 8.0
	6.74–6.77; m (2H)	6.77; d; 2.0	6.75; dd; 8.0, 1.5	6.78; d; 2.0
		6.80; d; 8.1	6.76; d; 1.5	6.82; dd; 8.0, 2.0
	6.82; d; 8.1 (1H)	6.88; d; 8.1	6.80; d; 8.0	6.85; d; 8.0

<b>Aromatic substituents</b>	3.76; s (3H, $\text{OCH}_3$ )	3.83; s (3H, $\text{OCH}_3$ )	5.93; s (2H, $\text{OCH}_2\text{O}$ )	3.86; s (3H, $\text{OCH}_3$ )
	3.79; s (3H, $\text{OCH}_3$ )	3.86; s (3H, $\text{OCH}_3$ )	5.98; s (2H, $\text{OCH}_2\text{O}$ )	3.87; s (3H, $\text{OCH}_3$ )
	0.14; s (6H, $\text{Si}(\text{CH}_3)_2$ )			5.91; s (2H, $\text{OCH}_2\text{O}$ )
	0.15; s (6H, $\text{Si}(\text{CH}_3)_2$ )			
	0.98; s (9H, $\text{Si}(\text{CH}_3)_3$ )			
	0.99; s (9H, $\text{Si}(\text{CH}_3)_3$ )			

<b>Ref.</b>	70	76	II	I
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